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Some Toxic Constituents of Chlorination-Stage Effluents from Bleached Kraft Pulp Mills[1]

A. B. McKAGUE
B.C. Research, Vancouver, B.C. V6S 2L2

McKAGUE, A. B. 1981. Some toxic constituents of chlorination-stage effluents from bleached kraft pulp mills. Can. J. Fish. Aquat. Sci. 38: 739–743.

Constituents responsible for the toxicity of a sample of bleached kraft chlorination-stage effluent to juvenile rainbow trout (*Salmo gairdneri*) were investigated. Tetrachlorocatechol, 3,4,5-trichlorocatechol, and 2,6-dichlorohydroquinone were identified and evidence was obtained for the presence of other chlorodihydroxybenzenes in toxic acidic fractions of the sample. Concentrations of 0.46 mg/L 3,4,5-trichloro- and 0.74 mg/L tetrachloro-catechol were estimated in the sample by analytical gas chromatography. Toxic materials in the nonacidic fraction were not identified although the nontoxic dichloromethyl methyl sulfone was isolated.

Key words: chlorination-stage effluent, toxicity, pulp mill, chlorocatechols, chlorodihydroxybenzenes

McKAGUE, A. B. 1981. Some toxic constituents of chlorination-stage effluents from bleached kraft pulp mills. Can. J. Fish. Aquat. Sci. 38: 739–743.

Les composés à l'origine de la toxicité d'un échantillon de l'effluent du stade de la chloration de la pâte kraft blanchie pour la truite arc-en-ciel (*Salmo gairdneri*) juvénile ont été étudiés. Le tétrachlorocatéchol, le trichloro-3,4,5 catéchol et la dichloro-2,6 hydroquinone ont été identifiés tandis qu'on obtenait la preuve de la présence d'autres chlorodihydroxybenzènes dans les fractions acides toxiques de l'échantillon. Par chromatographie en phase gazeuse, on a estimé que les concentrations de trichloro- et de tétrachlorocatéchol étaient respectivement de 0,46 et de 0,74 mg/L. Les substances toxiques de la fraction non

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[1] Based on work described in CPAR Report 245-6, 1979, Environmental Protection Service, Ottawa, Ontario.

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acide n'ont pas été identifiées, bien qu'on ait identifié la dichlorométhyl-méthyl-sulfone, non toxique.

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TOXIC constituents of pulp mill effluents have been the subject of extensive investigation over the past 10 years. Studies have been carried out on whole mill effluents and individual process systems with the result that most of the toxic constituents have now been identified. Acidic resin and fatty acids are major contributors to toxicity of a variety of effluents (Rogers et al. 1975; Leach and Thakore 1977). Trichloro- and tetrachloro-guaiacol are present in caustic extraction effluent (Rogers and Keith 1974; Leach and Thakore 1975) and a number of nonacidic diterpenoid alcohols have been identified in other effluents (Leach et al. 1977).

At the time when toxic constituents of most mill process streams were known, those of chlorination-stage effluents remained unidentified. While not as toxic as other individual process streams, toxic loadings from chlorination-stage effluents can be similar to or greater than caustic-stage effluents when discharge volumes are taken into account (A. Bruynesteyn and C. C. Walden unpublished data). Das et al. (1969) indirectly identified tetrachloro-*o*-benzoquinone and provided evidence for the presence of other quinones; however, little direct information regarding specific compounds was available. The purpose of this work was to identify the toxic constituents in chlorination-stage effluent from bleached kraft pulp mills.

Materials and Methods

GENERAL ANALYSIS

A combination of spectroscopic methods was used to identify compounds which were isolated during this study. Infrared (IR) spectra were obtained on a Perkin Elmer model 727 spectrophotometer, nuclear magnetic resonance (NMR) spectra were obtained on Varian HA 100 and XL 100 spectrometers, and mass spectra (MS) were obtained on Varian MAT CH4B (low resolution) and Kratos MS 50 (high resolution) mass spectrometers.

A Perkin Elmer model 3920 gas chromatograph equipped with a 0.25-mm ID × 15-m WCOT OV101 capillary column and flame ionization detector was used for analytical gas chromatography (GC). A linear gas flow of 30 cm/s helium and split ratios of 50 to 100:1 were used at the injector inlet. Column temperature was programmed from 100 to 200°C at 4 or 8°C/min for most analyses. A Varian 5700A gas chromatograph with a thermal conductivity detector was used to isolate small amounts of materials by preparative GC, and acidic materials were methylated with diazomethane before analysis by GC.

Column chromatography was done on Hi Flosil Silica Gel (60/200 mesh, Applied Science) and MN-Polyamide CC6 (Brinkmann), and precoated sheets of Silica Gel 60 F-254 (Brinkmann) and Polyamide 11 F-254 (Brinkmann) were used for thin-layer chromatography (TLC).

Static bioassays were performed with juvenile rainbow trout (*Salmo gairdneri*) in dechlorinated Vancouver tap water which had the following characteristics: pH 6.5, conductance 14.5 μs/cm, alkalinity 2.2 mg $CaCO_3$/L, EDTA hardness 4.55 mg $CaCO_3$/L, and temperature 11 ± 1°C. Fish loading was 0.5–2 g/L, dissolved oxygen content being maintained throughout the 96-h bioassay by gentle passage of air through Pasteur pipettes into the bioassay mixture.

The sample of chlorination-stage effluent was derived from an interior B.C. mill which was bleaching pulp from a 4:3:3 wood species mix of spruce:pine:fir. A C_DEHDED bleach sequence was being used at a temperature of 46°C and a Cl_2/ClO_2 of 8/0.3. Analytical data for the sample are given below.

Characteristics of the chlorination-stage sample

96-h LC50	~5%
pH	1.8
BOD_5 (mg/L)	425
TOC (mg/L)	442
Cl (g/L)	2.04
Cl_2	Not detected
Suspended solids (mg/L)	10
Total solids (mg/L)	1765
Tannin and lignin (mg/L)	99
Color (APHA)	288

ISOLATION OF TOXIC CONSTITUENTS

Three-litre columns of XAD-2 resin were washed with ether (6 L), methanol (6 L), and water (30 L), a sample of chlorination-stage effluent (120 L) was passed through the columns (30 L/column) at a rate of 3 L/h, and toxicity of the combined eluate from the columns was determined by bioassay as each 20-L portion was collected. Each column was eluted with ether (10 L) and methanol (3 L) and solvents were removed under reduced pressure to give an ether eluate and a methanol eluate. The ether eluate was dissolved in fresh ether (500 mL), filtered and combined with ether-soluble material obtained by washing the evaporated methanol eluate with ether (2 × 100 mL). After drying ($MgSO_4$) the ether eluate was re-evaporated. Residue from filtration of the ether eluate was combined with the methanol eluate and toxicity of the ether and methanol eluates was determined by bioassy.

A portion of the ether eluate equivalent to 25 L effluent (8.6 g) in ether (200 mL) was extracted with 10% NaOH (3 × 40 mL). The ether layer was washed with water (3 × 20 mL) and the washings combined with the NaOH extract. The ether layer was dried ($MgSO_4$) and evaporated to give a nonacidic fraction. The NaOH extract was cooled, acidified with concentrated HCl, and extracted with ether (5 × 40 mL). The combined extracts were processed in the usual manner to give an acidic fraction.

The acidic fraction from 25 L effluent (3.2 g) was separated into six fractions by chromatography on silica gel, fractions were collected with petroleum ether:benzene 1:1

(500 mL), benzene (500 mL), 2 × benzene:ether 1:1 (500 mL), and methanol (1.5 L), and toxicity of each fraction was determined.

A portion (280 mg) of fraction 4 (benzene:ether 1:1) was separated into 14 fractions on silica gel, solvent polarity was increased from benzene:ether 9:1 to 100% ether, and material remaining on the column was stripped off with methanol. After combination of similar fractions, components were methylated with diazomethane and small quantities of two compounds isolated by preparative GC on 5% 0V101. Material in fraction 4 was also chromatographed on polyamide, and fractions were collected with methanol.

A portion of fraction 3 was rechromatographed on silica gel and the column eluted with petroleum ether:benzene 1:1, benzene, and mixtures of benzene and ether. The major component was purified further by trituration with chloroform or by methylation and preparative GC on 5% 0V101.

Nonacidic material was fractionated by column chromatography on Silica Gel (weight ratio 60:1). Solvent polarity was increased from hexane:benzene 1:1 to methanol. A portion of the material in one of the fractions was purified further by preparative TLC. Small amounts of purified compounds were also obtained from some fractions by preparative GC on 5% 0V101.

Preparation of Reference Compounds

Samples of 4-chloro-, 4,5-dichloro-, 3,4,5-trichloro-, and tetrachloro-catechol were prepared by modified literature methods (Willstätter and Müller 1911). Tetrachlorocatechol was also prepared by reduction of tetrachloro-*o*-benzoquinone with sodium borohydride. Trichlorocatechol was purified by chromatography on polyamide and elution with methanol. The chlorinated catechols were crystallized from toluene. Dichlorohydroquinone (2,6-isomer) was prepared by reduction of 2,6-dichlorobenzoquinone with sodium borohydride in methanol, the product was triturated with chloroform and crystallized from methanol. Dichloromethyl methyl sulfone was prepared by chlorination of dimethylsulfide and oxidation of the product (Truce et al. 1952). For the estimation of 3,4,5-trichloro- and tetrachloro-catechol in the chlorination-stage sample, salt (100 g) was added to the effluent (1 L) and the sample extracted with ether (1 × 200 mL and 3 × 100 mL). The combined extracts were dried ($MgSO_4$), concentrated to 1 mL under reduced pressure, and methylated. The amounts of trichloro- and tetrachloro-catechol were estimated by comparison with standard solutions. A correction was made for recovery by extracting an aqueous solution of the two catechols in the same manner.

Results and Discussion

Effluent was nontoxic after passage through XAD-2 resin. Elution of the resin with ether yielded an eluate (41.5 g) which exhibited a toxicity curve similar to that of the original effluent (Fig. 1). The methanol eluate (24.1 g) was nontoxic when reconstituted to represent 100% original effluent concentration. These results showed toxicity was removed from the sample by XAD-2 resin and recovered by elution with ether.

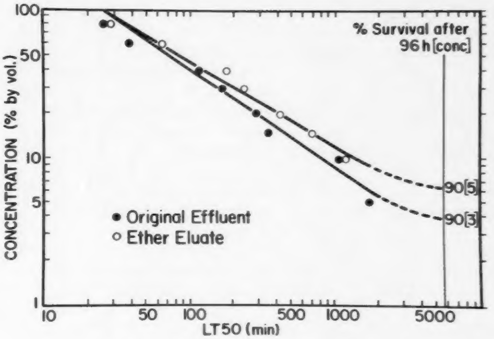

FIG. 1. Concentration-toxicity curves for chlorination-stage sample and corresponding ether eluate from XAD-2 resin. The lower portions of the curves were estimated on the basis that 90% survival was observed in the effluent at 3% concentration and in the ether eluate at 5% concentration.

Separation of a portion of the ether eluate equivalent to 25 L effluent (8.6 g) into acidic and nonacidic fractions resulted in a loss of 55% of the material, but little loss in toxicity. The acidic fraction contained 82% (3.2 g) of the recovered material; however, on the basis of LC50 values the nonacidic fraction was slightly more toxic than the acidic fraction.

Separation of the acidic material obtained above on Silica Gel resulted in substantial concentration of the toxicity into two fractions (Table 1). Fractions 3 and 4, eluted with benzene:ether 1:1, contained virtually all the toxicity.

Final purification of toxic material in fraction 4 was achieved by rechromatography on Silica Gel followed by preparative GC of material methylated with diazomethane. The two major components isolated were identified as 3,4,5-trichloroveratrole (I,$R=R'=CH_3$) and tetrachloroveratrole (II,$R=R'=CH_3$). TLC on Silica Gel (benzene:ethyl acetate 9:1) indicated chlorinated guaiacols, important toxic components of caustic extraction effluent (Leach and Thakore 1975), were absent. Therefore it was concluded that the parent compounds were the corresponding catechols (I,$R=R'=H$; II,$R=R'=H$). Chromatography of a portion of fraction 4 on polyamide, a method by which chlorinated catechols can be separated (Schwarz 1967), yielded

TABLE 1. Results from chromatography of the acidic fraction on Silica Gel

Fraction	Solvent	Weight material eluted (mg)	LT50[a] (min)
1	Petroleum:ether benzene 1:1	32	Nontoxic
2	Benzene	27	≈LC50
3	Benzene:ether 1:1	50	1244
4	Benzene:ether 1:1	477	51
5	Ether	156	Nontoxic
6	Methanol	1836	Nontoxic
	Total	2578	

[a] At 200% original effluent concentration.

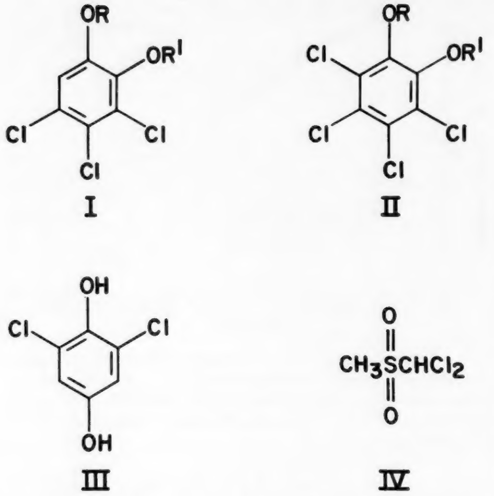

FIG. 2. Chemical structures of compounds identified in chlorination-stage sample.

3,4,5-trichlorocatechol and tetrachlorocatechol which gave IR spectra identical with those of synthetic samples.

Purification of the major component of fraction 3 was achieved by chromatography on Silica Gel. Elution with benzene yielded material which was obtained crystalline by trituration with chloroform. High resolution mass spectrometry established the molecular formula as $C_6H_4O_2Cl_2$; however, the compound was significantly less polar than 4,5-dichlorocatechol on TLC. Comparison of the melting point (158−160°C) and IR spectrum with those of other possible dichlorodihydroxybenzenes established the identity as 2,6-dichlorohydroquinone (III).

The three major chlorodihydroxybenzenes identified in the acidic fraction were the major components of the toxic fractions 3 and 4 (Table 1). Smaller amounts of at least three additional components with TLC properties similar to those phenols identified above could be detected. These are likely isomeric chlorodihydroxybenzenes.

Fractionation of nonacidic material by Silica Gel chromatography yielded a series of fractions which were still mixtures of compounds. High resolution mass spectrometry of one compound purified by preparative GC revealed it had a molecular formula of $C_2H_4Cl_2O_2S$. This compound was identified from its NMR and IR spectra as dichloromethyl methyl sulfone (IV) and was identical with material prepared by chlorination and oxidation of dimethylsulfide, a possible route to formation during bleaching in the mill. Dichloromethyl methyl sulfone was nontoxic up to 10 mg/L. Spectral data were obtained on some additional nonacidic compounds; however, these were not identified so the nature of the toxic constituents in the nonacidic fraction remains unknown.

Approximate LC50 values for various chlorodihydroxybenzenes are shown in Table 2. Although not isolated, 4,5-dichlorocatechol is included since evidence indicated the presence of other chlorodihydroxybenzenes. Both Das et al. (1969) and Servizi et al. (unpublished data) reported chlorinated catechols were highly toxic to salmon. The dichlorohydroquinone was more toxic than any of the chlorocatechols.

TABLE 2. Toxicity of chlorodihydroxybenzenes to juvenile rainbow trout, *Salmo gairdneri*

Compound	LC50 (mg/L)
4,5-Dichlorocatechol	0.5<LC50<1.0
3,4,5-Trichlorocatechol	1.0<LC50<1.5
Tetrachlorocatechol	1.5
2,6-Dichlorohydroquinone	<0.5

The concentrations of 3,4,5-trichloro- and tetrachlorocatechol were estimated by GC of methylated ether extracts of the sample under study to be 0.46 mg/L and 0.74 mg/L, respectively. The concentration of 2,6-dichlorohydroquinone was not estimated because methylation with diazomethane proceeded at a very slow rate and was probably incomplete. Since the LC50 values for the various chlorodihydroxybenzenes shown in Table 2 are in the range of 0.5−2.0 mg/L, toxicity of the acidic fraction of the sample of chlorination-stage effluent can likely be accounted for by the combined quantities present.

At the time this study was performed the toxic constituents of chlorination-stage effluent were unknown. Apart from the aforementioned work by Das et al. (1969), reports in the literature discussed general characteristics of components produced during chlorination (Erickson and Dence 1976; Simson et al. 1978) but did not involve actual isolation or correlation of specific compounds with toxicity. Lindström and Nordin (1976) reported the presence of isomeric trichlorocatechols and tetrachlorocatechol in chlorination-stage effluent at concentrations below the threshold for toxicity. Since the time of the work described in this paper, other studies have been performed (S. Kachi et al. unpublished data; R. H. Voss et al. unpublished data) implicating chlorocatechols as toxic components of chlorination-stage effluent.

Although the present work demonstrates that chlorodihydroxybenzenes probably account for the toxicity of the acidic fraction of this sample, the identity of toxic components in the nonacidic fraction is not known. Some progress was made toward purification of the nonacidic materials, and the identification of dichloromethyl methyl sulfone as well as the report by Lindström and Nordin (1978) suggest a wide variety of nonacidic compounds is present.

Acknowledgments

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Salmon Stock Identification Using a Microanalytic Technique to Measure Elements Present in the Freshwater Growth Region of Scales

LOUIS A. LAPI AND T. J. MULLIGAN

Department of Fisheries and Oceans, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

LAPI, L. A., AND T. J. MULLIGAN. 1981. Salmon stock identification using a microanalytical technique to measure elements present in the freshwater growth region of scales. Can. J. Fish. Aquat. Sci. 38: 744–751.

Scanning electron microscopy combined with energy dispersive X-ray analysis was used to measure the chemical composition of the freshwater growth region of salmonid scales. Adult sockeye salmon (*Oncorhynchus nerka*) scales from three lakes on the west coast of Vancouver Island were analyzed and the data were subjected to multivariate statistical analysis. The fish from the three lakes were found to be chemically distinct, enabling 63% of the fish to be correctly classified to their lake of origin. This stock identification technique can differentiate populations, allows reduced sample size, and requires no handling of juvenile fish.

Key words: stock identification, salmon, X-ray analysis, scanning electron microscopy, multivariate analysis

LAPI, L. A., AND T. J. MULLIGAN. 1981. Salmon stock identification using a microanalytical technique to measure elements present in the freshwater growth region of scales. Can. J. Fish. Aquat. Sci. 38: 744–751.

La composition chimique de la région de croissance en eau douce d'écailles de salmonidés a été déterminée par microscopie électronique à balayage couplée à une analyse par rayons X à énergie dispersive. Nous avons mené cette étude sur des écailles de saumons nerka (*Oncorhynchus nerka*) provenant de trois lacs de la côte occidentale de l'île Vancouver, et soumis les données à une analyse multidimensionnelle. Nous constatons que les écailles des poissons des trois lacs sont chimiquement distinctes, de sorte qu'il a été possible d'assigner correctement 63% des poissons à leur lac d'origine. Cette méthode d'identification des stocks permet de séparer des populations, de réduire la taille des échantillons et d'éviter la manipulation des juvéniles.

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CONTINUED maximum yield and optimim quality are the goals of Pacific salmon management strategy. To fulfill these objectives each functional production unit must be defined and managed separately. Because of the Pacific salmon's anadromous life cycle in which it returns to its natal site, each stream or major tributary that contains a reproducing population may be considered a separate stock or functional production unit. Ideally each population should be managed individually; however, since a major portion of the salmon fishery occurs on mixed stocks in salt water, individual stock management is not possible unless fish can be identified by stock at the time the fishery is taking place.

Classically identification has been accomplished by external marks such as finclipping and branding, or by pigmented dyes and coded wire tags. In recent years investigators have looked at chemical differences as a means of separating stocks of fish. This type of analysis is based on the premise that geographically distinct populations reflect the chemical constituents of the water in which they reside. Klokov and Frolenko (1970) found chemical differences in sclaes from three stocks of pink salmon (*Oncorhynchus gorbuscha*) from the Kamchatka Peninsula. Neutron activation analysis was used by the Fisheries Agency of Japan (1967) to identify different stocks of chum salmon (*O. keta*). To explore more sensitive and broader-based analyses, Calaprice (1971) used X-ray fluorescence spectroscopy and successfully separated stocks of sockeye salmon.

The greatest chemical difference in the environment may be encountered during the Pacific salmon's freshwater residence when stocks are separated geographically. Since the chemical composition of different streams might be expected to be very different, and since it is known that fish have the ability to concentrate chemical elements from the environment, it was anticipated that the greatest differences would occur between stocks originating from dissimilar freshwater environments.

The ideal tissue for analysis is one containing a separable record of the time spent in freshwater and salt water. Scales

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approximate this ideal, since they are laid down concentrically so that the central portion contains elements incorporated during the freshwater life of the fish. Scales are metabolically inert during most of the fish's life; therefore, the elemental concentrations should remain relatively unchanged.

This objective of this research was to determine if the natural elemental composition of the freshwater growth region of individual scales, as measured by the scanning electron microscope (SEM) and X-ray microanalysis, could be used to separate Pacific salmon into distinct populations.

Materials and Methods

Scanning electron microscopy (SEM) in conjunction with X-ray energy dispersive microanalysis was chosen as the analytic method. SEM can utilize almost the entire freshwater area of the scale; it simultaneously analyzes all elements with atomic number ≥11; it is nondestructive; and it can analyze over a wide range of elemental concentrations (from ~100 mg/g to 100%). The technique compares favorably to laser beam microprobe analysis which can only look at a small fixed area, is sensitive over a small range of concentrations, and cannot analyze as many elements simultaneously.

SEM uses the electron beam of the microscope to excite the elements from a specific location in a sample. When electrons in the beam strike the sample they can interact to produce X rays in two ways: by scattering, which results in a continuum spectrum (X rays of a continuous energy), or by inner atomic shell ionization, which results in production of a characteristic or discrete spectrum (X rays of exact energy). Figure 1 shows a typical X-ray microanalysis spectrum of a sockeye salmon (*O. nerka*) scale composed of peaks produced by characteristic X rays superimposed on the continum spectrum.

Sockeye salmon from Great Central Lake, Sproat Lake, and Henderson Lake were chosen for the study because all fish spend at least 1 year in freshwater, and samples were easily obtained. Together these three lakes form the basis of a fishery in Barkley Sound (49°N, 125°W) and represent a typical problem in fisheries management. In the fishery, fishermen take the fish without knowledge of their lake of origin. As a result one lake may receive too few spawners and another lake may have too many spawners, resulting in lowered production for both lakes.

Sixty-four fish from Sproat River were collected on July 7, 1972, in a trap at the head of a low water fishway 1.6 km from Sproat Lake and 6.4 km from the Stamp River. Forty-six samples were obtained in a trap on August 3, 1972, at the head of a fish ladder on the Stamp River near the mouth of Great Central Lake. Forty-four spawners were taken from Henderson Lake with a gill net September 5–6, 1972. Several scales were taken from the preferred area of each fish (below the dorsal fin, two scales above the lateral line, on the fish's left side), and stored in individual 28-mL vials in a 50% glycerin and distilled water solution.

Prior to microanalysis the scales were immersed in distilled water and cleaned in a sonic bath until all signs of foreign material had been removed. Individual scale samples were mounted on spectroscopically pure carbon specimen holders using a commercial glue (LePage's Glue-All), then coated with 10 to 15 nm of carbon in a vacuum evaporator. The thickness of the coating was determined by placing a chip of porcelain, with a drop of diffusion pump oil on it, in the evaporator. When a color difference between the oil drop and

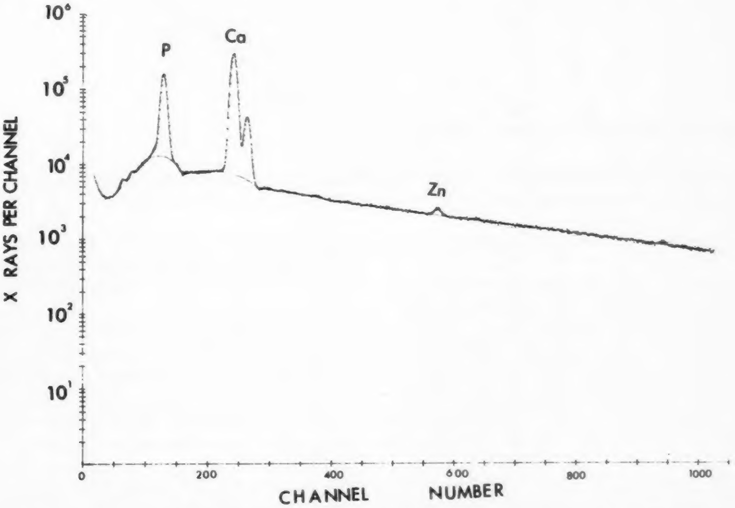

FIG. 1. The X-ray spectrum of the freshwater portion of sockeye salmon scales. Energy of X-ray is proportional to channel number. Major peaks in the spectrum have been identified by the chemical symbol of the element which produced them.

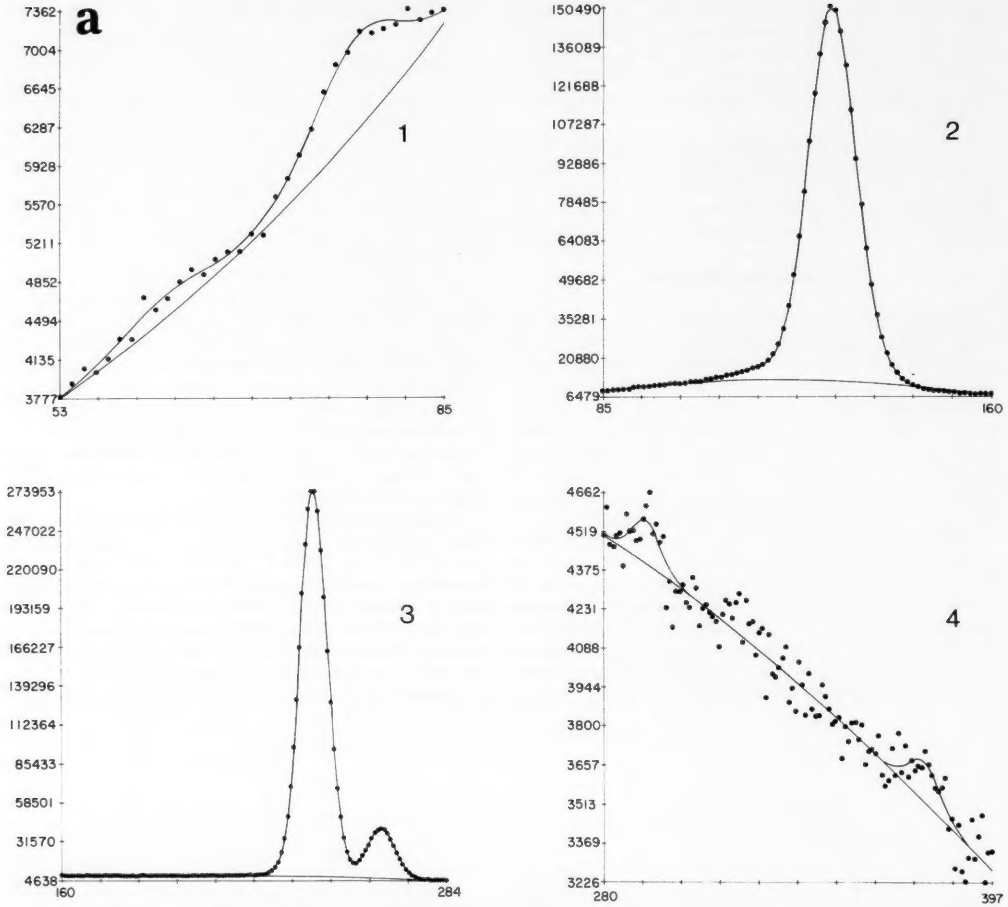

FIG. 2. (a) Detail of X-ray spectrum of freshwater region of sockeye salmon scale showing data points and least squares fit. (1) Na and Mg, (2) phosphorus, (3) calcium, (4) barium peak and peak due to "pile up" of two, and calcium X-rays.

the rest of the porcelain was detected the coating procedure was stopped.

The SEM was turned on and allowed to stabilize for 30 min before the specimen was inserted. The sample was tilted to a 45° angle with respect to the incident electron beam and centered in the view of the X-ray detector so that the desired portion of the scale (the freshwater zone) was exposed to the electron beam and the maximum X-ray count rate obtained. The current was then adjusted to obtain the optimum count rate (3000 counts per second). The rate was noted and the stage of the SEM was moved so that the beam was exposed to the Faraday cup (a small brass can having a 1-mm hole in one end). The magnification was increased until the entire beam went through the hole in the cup, and was conducted; to ground through the microscope stage. A current meter attached to the stage of the SEM then measured the true incident beam current. The stage was immediately repositioned to the chosen sample location, the magnification reduced to its original value, and a second count rate measurement was taken. If no change was observed between the two count rate measurements, the beam current was assumed to have been constant and the initial current and count rate were recorded (Mulligan and Lapi 1976).

An aluminum collimator designed for the X-ray detector effectively eliminated the scattered radiation. An added advantage of the collimator was to fix the X-ray takeoff angle at 72° (the takeoff angle is the angle at which the X ray leaves the samples relative to the horizontal). Yakowitz (1974) shows that such a high takeoff angle helps to eliminate the effect of surface irregularities and improves the repeatability of analysis.

Spectra were broken up into a series of sections. Guassian

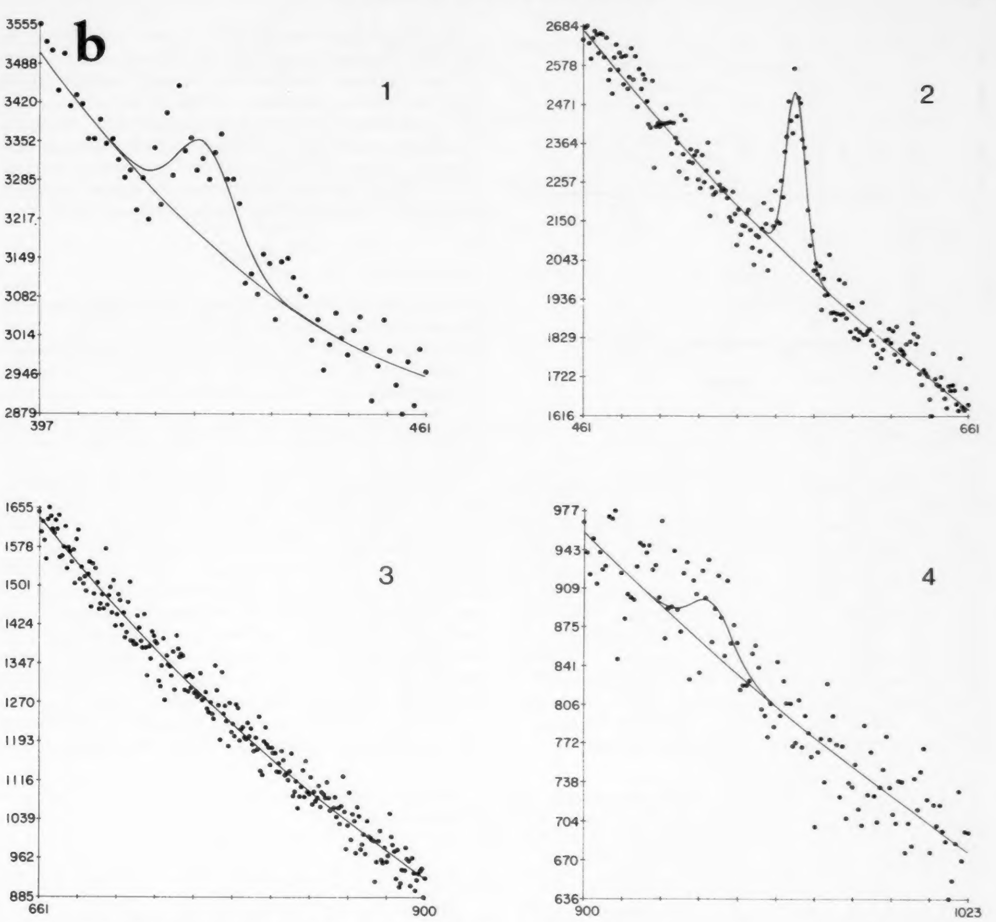

FIG. 2. (b) Detail of X-ray spectrum of freshwater region of sockeye salmon scale showing data points and least squares fit. (1) Iron, (2) zinc, (3) no peaks, (4) strontium.

distributions were fit to the X-ray peaks while the continuum was fit using a polynomial or exponential expression (Fig. 2a and 2b). A least-squares method was used to determine the best fit (Bevington 1969).

Samples were run in random order among the three stocks. Each sample was counted for ~$1\frac{1}{2}$ h. To compare spectra, peaks were normalized to give peak area in terms of X rays per second per microcoulomb of the electron beam current. Normalization was accomplished by the formula

$$A_n = \frac{A \times R}{T \times i}$$

where A_n = normalized area, A = peak area calcuated from the fit, T = total number of X rays in the entire spectrum, i = beam current, and R = count rate. Errors were calculated using $\Delta A = \sqrt{A_n^2 + (B_n^2 \times \chi^2)}$ where ΔA = one standard deviation of peak area, A_n = normalized area of the peak, B_n = normalized area of the background, and χ^2 = chi square for the fit of that particular section of the X-ray spectrum. The results of this normalization for the Cu peak in a brass standard spectrum, run repeatedly over a period of several months, are shown in Fig. 3. This figure demonstrates the high degree of repeatability possible when the normalization procedure is used. The mean error in the normalized Cu peak in the spectrum from the brass standard was (1.5% ± 1%) over an elapsed time of ~6 mo. For peak areas from scale spectra, the normalization procedure yielded repeatable results within ±5% for the major peaks such as P and Ca. Lesser peaks, such as Ba, Fe, and Zn, were more affected by the analytic error (represented by the square root of the total peak area for Poisson counting statistics) than by the

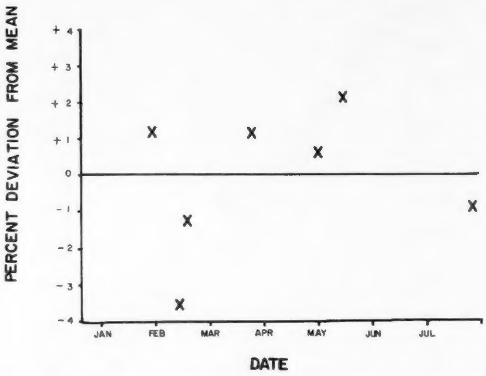

FIG. 3. Percent deviation of normalized peak area for brass standard versus time.

error introduced through uncertainties in the normalization procedure.

Quantitative X-ray microanalysis was not attempted for the following reasons: all methods of quantitative X-ray microanalysis require samples to have smooth flat surfaces (Yakowitz 1974). Because of the presence of circuli, or growth rings, scales do not meet this criterion. Quantitative procedures, such as the ZAF corrections prepared by the National Bureau of Standards, are unsuccessful in light element matrices (Yakowitz 1975) as a result of fluorescence of X rays by the continuum spectrum. Scales are mostly calcium phosphate and organic compounds; therefore, the matrix is too light for standard quantitative analysis procedures.

Normalized peak areas for each element as measured for each fish were used as variables in the stock discrimination analysis. Stepwise discriminant function analysis (Dixon 1970) was used to determine whether or not differences in population means of samples from each lake were statistically significant, and to attempt to classify individual fish as to their stream of capture. As a complement to discriminant analysis, cluster analysis was used to determine the number of natural grouping or clusters of observations (Veldman 1967).

Results

A high degree of overlap among the three populations for any one element is evident from examination of Table 1. Even the elements with the greatest differences among populations, i.e. Zn and Ba, show considerable overlap. Multivariate analysis techniques were chosen because they can sometimes separate different populations of observations when each measurement taken individually overlaps among the populations.

The statistical significance associated with the null hypotheses that the group mean vectors (vectors in the *n*-dimensional space which point to the group means) are equal was less than 0.0001. The stepwise discriminant analysis had eight variables, the elements found in Table 1, for each observation presented to it. The first variable selected by the program was zinc, which was responsible for the separation of Sproat Lake from Great Central Lake and Henderson Lake fish, but could not separate Great Central Lake fish from Henderson Lake fish (Table 2). The next variable selected as barium. This reduced the probability for group mean vectors for Great Central Lake and Henderson Lake fish being drawn from the same population to 0.0001 and increased the percent of indi-

TABLE 1. Means of normalized peak areas and standard deviations for elements found in fish from Great Central, Sproat, and Henderson lakes.

Element	Great Central Lake fish	Sproat Lake fish	Henderson Lake fish
Na	3 148±2 135	3 172±1 852	2 377±1 021
Mg	8 519±2 749	7 807±3 153	8 955±3 478
P	791 690±48 948	786 880±53 661	775 500±48 023
Ca	1 657 300±75 348	1 656 800±111 430	1 675 500±55 843
Ba	783±423	1 009±717	1 624±795
Zn	2 585±831	4 531±2 160	3 030±1 338
Sr	496±115	520±164	559±143

TABLE 2. Probabilities of samples being drawn from the same population.

	Population	
Population	Great Central Lake fish	Sproat Lake fish
After step 1 of discriminant analysis (discrimination based on Zn)		
Sproat Lake fish	0.00001	
Henderson Lake fish	0.27136	0.00036
After step 2 of discriminant analysis (discrimination based on Zn and Ba)		
Sproat Lake fish	0.00003	
Henderson Lake fish	0.00004	0.00001

TABLE 3. Percentage of fish classified as being from a particular lake.

Known population	Classified population Great Central Lake	Sproat Lake	Henderson Lake
After step 1 (discrimination based on Zn) 57.61% correctly classified			
Great Central Lake fish	76.63	6.67	16.67
Sproat Lake fish	18.75	53.13	28.13
Henderson Lake fish	43.33	13.33	43.33
After step 2 (discrimination based on Zn and Ba) 63.04% correctly classified			
Great Central Lake fish	76.67	6.67	16.67
Sproat Lake fish	25.00	46.88	28.13
Henderson Lake fish	23.33	10.00	66.67

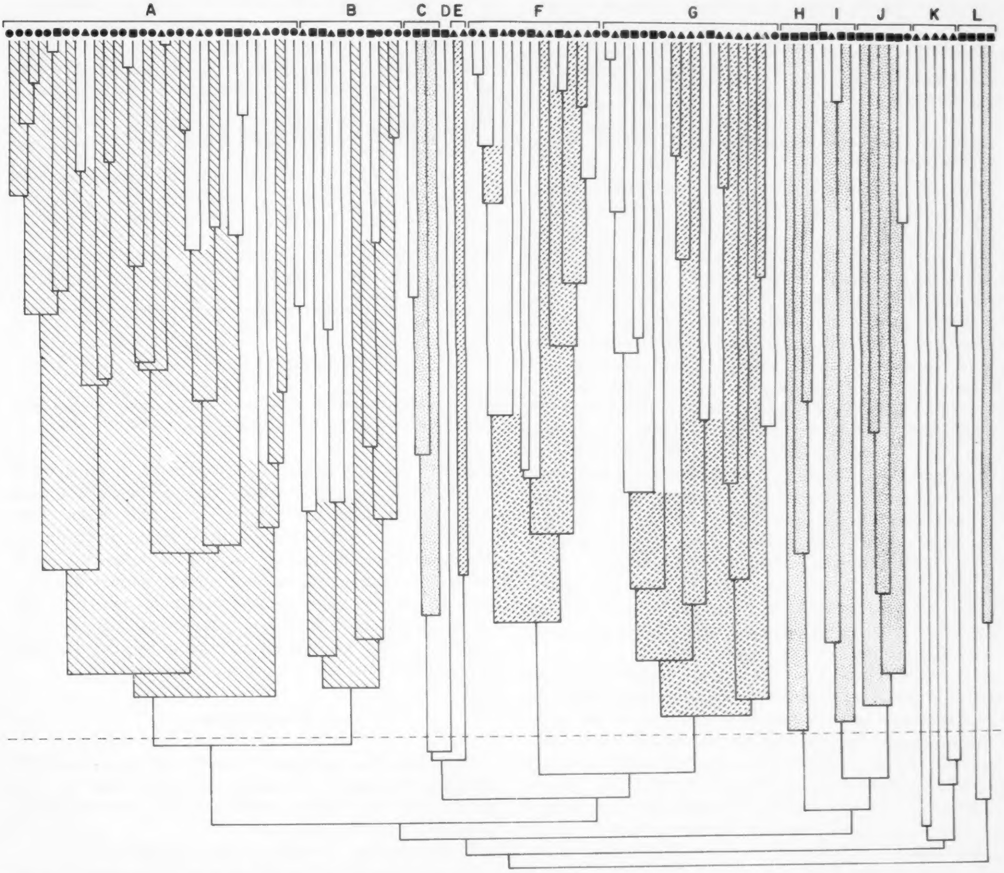

FIG. 4. Cluster analysis of fish based on zinc and barium abundance in freshwater component of sockeye salmon scales. Areas not shaded are samples not jointed to samples from the same lake or clustered after marked increase in clustering distance. ●, Great Central Lake fish; ■, Sproat Lake fish; ▲, Henderson Lake fish; ▧, clustering of Great Central Lake fish; ░, clustering of Sproat Lake fish; ▩, clustering of Henderson Lake fish; ---, large increase in clustering distance.

viduals classified to their correct group from 57.6 to 63% (Table 3). All other variables were not significantly different at the 0.95 confidence level.

Cluster analysis using all eight variables showed little if any clustering by population. This result was most likely due to the presence of nonsignificant variables, which tend to mask out the important variables. Figure 4 presents a dendogram depicting the results of the cluster analysis using only Zn and Ba as variables. On the horizontal axis in this figure is plotted each individual observation (fish). The vertical axis is nonlinear and represents the similarity between observations. When two observations are joined by the lines represented in the dendogram, they now form a cluster which can be joined with other individual observations or clusters. Thus the analysis joins the most similar fish into cluster, based on their Ba and Zn content, without regard to the stock. The horizontal dashed line near the bottom of the figure defines the point on the vertical axis at which the similarity between objects being clustered has decreased dramatically. Therefore, clusters A–L are defined as those formed by the analysis before this dramatic step.

Discussion

The data demonstrate that fish scales from Sproat Lake tend to have a higher zinc concentration than fish scales from the other two lakes. There was, however, considerable overlap among these three populations even in the two-dimensional space of the normalized peak areas for Zn versus Ba (Fig. 5). This figure shows a relatively wide range in Zn concentration for Sproat Lake fish compared to Henderson and Great Central Lake fish. This large range in values for Sproat Lake fish is responsible for much of the misclassification and could be due to factors such as straying of fish from other lakes, or the presence of more than one population in the lake. The average analytic error in the measurement of the Zn peak areas, 16%, is much less than the standard deviation for any of the populations (Table 1) and was probably not responsible for very much misclassification of individual fish. The average analytical error in measurement of the Ba peak areas was large, 59%, due to its low concentration in the scales and may be responsible in part for the large standard deviation of Ba in the three populations. Elements such as Sr were not found to be important in separating populations. Because of their low concentration, the analytic error may override any differences among populations.

One of the drawbacks of discriminant analysis is that the number of groups or stocks must be known beforehand. If a lake has two or more distinct populations, problems arise. Cluster analysis, since it does not assume prior knowledge about the number of populations, but looks only at natural grouping, may avoid some of these problems. Figure 4 shows 12 groups (A–L), nine clusters, and eight individuals remaining unclustered (D, K, and L). Clusters A and B contain mostly Great Central Lake fish; clusters C, H, I, and J contain mostly Sproat Lake fish; and clusters E, F, and G contain mostly Henderson Lake fish. Cluster K contains individuals not joined but belonging to Henderson Lake whereas cluster L contains samples from Sproat Lake that were not joined. By looking futher at the clustering, it appeared that Sproat Lake cluster C was very dissimilar to clusters H, I, and J which eventually are joined together, whereas the clusters from Great Central Lake join together as do the clusters from Henderson Lake. This dissimilarity in Sproat Lake clusters and the difficulty in assigning individuals to Sproat Lake by the discriminant analysis method support the idea that there may be two dissimilar populations in Sproat Lake. The spawning population is divided into lake spawners and river spawners, which gives some credence to the idea that there may be more than one stock in the lake.

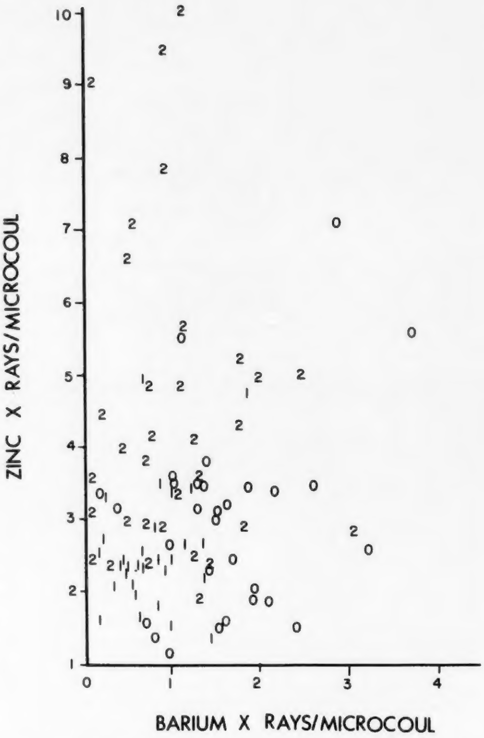

FIG. 5. Zinc vs. barium abundance in scales from samples. 0 = Henderson Lake, 1 = Great Central Lake, 2 = Sproat Lake.

Conclusions

Stock identification using scanning electron microanalysis compares favorably with many techniques tried on wild stocks, such as branding, coded wire tags, and finclipping. In general the advantages are:

1) No handling of smolts or fry is required.
2) Sampling is much easier since all fish collected can be analyzed instead of searching for marked fish.
3) Recovery of enough marks is not a problem.

Although coded wire nose tags have been very successful tagging hatchery stock, marking wild smolts has not been very successful. For example, of 17 849 coho smolts tagged in 1972 on the Squamish River, only 27 tags were recovered (Wilson et al. 1977).

There are still many difficulties with stock discrimination based on scanning electron microanalysis. It is not 100% accurate and a long time to analyze each sample is required ($1\frac{1}{2}$ h just to obtain the X-ray spectrum). Because of the sensitivity limits of microanalysis, many elements were not detected in every sample or had such high analytic uncertainty that they were not useful for population separation. If the sensitivity could be increased, then more elements might become useful for separating populations, thus increasing the percentage of individuals correctly classified.

The objective of Pacific salmon management requires that fish taken in a mixed fishery can be identified as belonging to a particular stock. As yet no reliable method has been found to determine the stream of origin other than tagging or finclipping, methods which are too costly to be used other than on specific projects. Scanning electron microanalysis shows promise as an alternative. It differentiates populations; reduces sample size; and requires no handling of smolts.

Acknowledgments

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Comparison of Ozone and Chlorine Toxicity to the Developmental Stages of Striped Bass, *Morone saxatilis*[1]

LENWOOD W. HALL JR.,[2] DENNIS T. BURTON,[2] AND LEONARD B. RICHARDSON[2]

The Academy of Natural Sciences of Philadelphia, Benedict Estuarine Research Laboratory, Benedict, MD 20612, USA

HALL, L. W. JR., D. T. BURTON, AND L. B. RICHARDSON. 1981. Comparison of ozone and chlorine toxicity to the developmental stages of striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 38: 752–757.

Toxicity of ozone-produced oxidants (OPO) to striped bass, *Morone saxatilis*, eggs, larvae, and fingerlings was determined under continuous-flow conditions. Eggs, tested in both fresh and estuarine water, were found to be significantly ($P < 0.001$) more sensitive to OPO in freshwater. The higher sensitivity found for striped bass eggs in freshwater suggests that ozone could have a more pronounced effect if discharged in freshwater industrial or municipal wastewaters located in the vicinity of a striped bass spawning area. The ozone data collected in this study were compared with previously published chlorine toxicity data for each striped bass life stage. The toxicity of chlorine and ozone was found to be similar with striped bass eggs and larvae in estuarine water. LC50's for fingerlings were 0.20 mg/L OPO at 6 h and 0.08 mg/L OPO at 96 h.

Key words: ozone, oxidants, chlorine, striped bass, *Morone saxatilis*; wastewaters

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La toxicité des oxydants dérivés de l'ozone(ODO) pour le bar d'Amérique, *Morone saxatilis*, ses oeufs, ses larves et ses alevins a été déterminée dans des conditions de débit continu. Lors d'essais avec de l'eau douce et de l'eau d'estuaire, on a constaté que les oeufs étaient nettement plus sensibles ($P < 0{,}001$) aux ODO dans l'eau douce. Ce fait donne à penser que l'ozone aurait un effet très puissant s'il était présent dans les eaux usées industrielles ou municipales déversées dans un cours d'eau à proximité d'une frayère de bar. Les données sur l'ozone recueillies au cours de l'étude ont été comparées à des données sur la toxicité du chlore pour le bar à chaque étape de sa vie, qui avaient été publiées auparavant. On a constaté que le chlore et l'ozone avaient une toxicité similaire dans les eaux d'estuaire pour les oeufs et les larves du bar. Les CL50 des ODO pour les alevins se chiffraient après 6 h et 96 h respectivement, à 0,20 et 0,08 mg/L.

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IN recent years chlorine has been widely used for disinfection of municipal and industrial wastewaters (Jolley et al. 1978). Although this biocide and its by-products are an effective disinfectant, its toxic effects on aquatic biota (Opresko and Hannon 1979; Hall et al. 1981b) and possible carcinogenicity to man (Jolley 1973) have caused concern. Several strong oxidizing agents such as bromine, bromine chloride, chlorine dioxide, hydrogen peroxide, iodine, and ozone have been suggested as alternatives to chlorine (Burton and Liden 1978). Ozone appears to be one of the most viable alternatives to chlorine for water and wastewater treatment (Evans 1972). The advantages of using ozone in place of chlorine in freshwater systems are (1) inorganic salt content is not increased after reaction; (2) taste or color of treated water is not affected; (3) it is a more powerful disinfectant; (4) undesirable residual effects may be eliminated; (5) it rapidly decomposes in water, and (6) it is generated on site thus reducing storage problems and assuring supply at a known price (Marine Research Inc. 1976).

Ozone toxicity to freshwater fish has been documented by various investigators (Frese 1974; Arthur and Mount 1975; Arthur et al. 1975; Rosenlund 1975; Le Paulone 1975; Wedemeyer et al. 1979; Asbury and Coler 1980); however, investigations on estuarine fish are limited (Toner and Brooks 1975; Block et al. 1978). This study was designed to (1) evaluate the effects of ozone on striped bass, *Morone saxatilis*, eggs, larvae, and fingerlings; (2) compare the toxicity of ozone with previously published chlorine toxicity data for

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[2] Present address: The Johns Hopkins University, Applied Physics Laboratory, Aquatic Ecology Section, Shady Side, MD 20867, USA.

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each striped bass developmental stage, and (3) compare the toxicity of ozone to eggs in fresh and estuarine water. Striped bass were selected for this study because they are anadromous fish with spawning grounds sometimes located in the vicinity of industrial and municipal facilities which may discharge large amounts of biocides.

Materials and Methods

GENERAL PROCEDURES

Water-hardened eggs of fertilized striped bass, *M. saxatilis*, were obtained from Brookneal Striped Bass Hatchery (freshwater) in Brookneal, Virginia, and transferred to the laboratory in sealed plastic bags with oxygen-saturated water at ~18°C. Eggs were maintained in both estuarine (3.5°/oo) and freshwater in 12-L hatchery jars. Larvae were reared in estuarine water from the egg stock to a total length of ~20 mm in hatchery trays at densities <1 g/L. Larvae were fed natural plankton introduced via the estuarine system and supplemented with high densities of brine shrimp, *Artemia salina*. Fingerlings (≃13 cm total length), obtained from a hatchery operation at Ichthyological Associates Incorporated in Middletown, Delaware, were acclimated for 14 d before testing. These organisms were fed precooked commercial shrimp twice daily during the acclimation period. The range of various water quality parameters during each study is listed in Table 1. Photoperiods for the acclimation and testing of all life stages were maintained at natural light (L)−dark (D) conditions which were ~12 h L:12 h D for eggs and larvae and 14 h L:10 h D for fingerlings.

EXPERIMENTAL PROCEDURES

The water used in these studies was deep well water and estuarine water pumped into the laboratory from the Patuxent River, Maryland (~33 km from its outlet at Chesapeake Bay).

The effects of ozone-produced oxidants (OPO) to 12-h-old striped bass eggs were evaluated by continuously exposing this life stage to 0.00, 0.005, 0.01, 0.05, and 0.10 mg/L OPO (as equivalent total residual chlorine: TRC). Eggs in both fresh and estuarine water (Table 1) were exposed to OPO concentrations for periods of 12 and 30 h; hatch occurred when eggs were ~48 h old. Eggs were tested in both fresh and estuarine water because striped bass typically spawn in freshwater while the developing eggs and prolarvae are exposed to increasing salinities as they are transported down the estuary. All eggs were tested in cylindrical PVC test chambers (volume 206 mL) fitted with 350-μm nylon mesh placed in 7-L hatchery trays. Three sets of 30 eggs each were exposed to each test concentration. Mortality observations were recorded at the end of each exposure period. The criteria for death were a change from a normal transparent color to an opaque color and/or failure to hatch.

Striped bass larvae were subjected to continuous OPO concentrations of 0.00, 0.01, 0.05, 0.10, 0.15, 0.20, and 0.30 mg/L for periods up to 96 h. Forty larvae were tested at each concentration in 7-L hatchery trays. Mortality observations, water quality, and OPO concentrations were recorded at 6, 12, 24, 36, 48, 60, 72, 84, and 96 h. The criteria used for death of the larvae were cessation of opercular movement and/or lack of movement in response to a mild mechanical stimulus.

TABLE 1. Ranges of water quality during the striped bass egg, larvae, and fingerling studies. Egg studies were conducted in both estuarine and freshwater.

Life stage	Temp. (°C)	Salinity (°/oo)	pH	Dissolved oxygen (mg/L)
Eggs	17.4−17.5	3.5−3.6	7.7−7.8	6.9−9.0
Eggs	18.9−19.0	0	7.5−7.6	6.0−8.5
Larvae	22.8−23.1	11.1−11.3	7.6−7.7	6.0−9.5
Fingerlings	25.0−25.3	9.5−10.2	7.0−7.4	6.3−9.6

Ozone-produced oxidant effects on striped bass fingerlings were assessed by continuously exposing test organisms to OPO concentrations of 0.00, 0.01, 0.05, 0.075, 0.10, 0.20, 0.30, 0.40, and 0.50 mg/L for periods up to 96 h. Twenty organisms were tested at each concentration in 57-L glass aquaria. Mortality observations, water quality data, and OPO concentrations were recorded at 6, 12, 24, 48, 60, 72, and 96 h. Death criteria used for fingerlings were similar to those used for larvae.

Ozone gas was produced with an electrical discharge type ozonator (model T-816; Welsbach Ozone System Corp., Philadelphia, PA) using dry aviator oxygen. Ozonated oxygen (≃4% O_3 by volume) was bubbled into the base of an 11.5-L polycarbonate counter current contact column (2.75 m height, 7 cm diameter) at 1.5 L/min through a 3-cm-fritted glass disc. Filtered Patuxent River water was pumped into the top of the column; ozonated water was drawn from the lower part of the column and fed through glass rotometers to head tanks. Filtered water and appropriate OPO concentrations were mixed in the head tanks to form the various study concentrations and delivered in continuous flow at 4 L/min through 13 mm PVC tubing to the vessels in which the tests were conducted.

Ozone-produced oxidant concentrations (±0.002 mg/L) were measured amperometrically during exposure as equivalent total residual chlorine (American Public Health Association, American Water Works Association and Water Pollution Control Federation 1976) every 6 h during the study using a PAR polarographic analyzer (model No. 174A; Princeton Applied Research Corp., Princeton, NJ). Other water quality data were also recorded at this time interval (See Table 1).

STATISTICAL PROCEDURES

The toxicity data for all striped bass life stages are presented in Table 2. Analysis of these data involved estimation of LC50 values, derivation of percent mortality predictive equations (Table 3), development of percent mortality response surfaces for larvae and fingerling (Fig. 1 and 2), and comparisons of sensitivity between striped bass eggs in fresh and estuarine water (Table 4). LC50 values for freshwater eggs (probit LC50's could not be calculated for estuarine eggs), estuarine larvae, and fingerlings were estimated by the

TABLE 2. Toxicity data for striped bass eggs, larvae, and fingerlings. The cumulative percent mortality is reported for each OPO concentration, although replicates were used with the eggs.

Life stage	Exposure period (h)	Cumulative percent mortality at OPO concentrations (mg/L)										
		0.00	0.005	0.010	0.050	0.075	0.100	0.15	0.20	0.30	0.40	0.50
Freshwater eggs	12	3.3	5.6	15.6	50.0	a	70.0	a	a	a	a	a
	30	6.7	5.6	25.6	43.3	a	80.0	a	a	a	a	a
Estuarine eggs	12	7.8	4.4	13.3	1.1	a	23.3	a	a	a	a	a
	30	3.3	3.3	5.6	6.7	a	8.9	a	a	a	a	a
Estuarine larvae	6	5.0	a	2.5	2.5	a	2.5	100.0	100.0	100.0	a	a
	12	5.0	a	2.5	2.5	a	5.0	100.0	100.0	100.0	a	a
	24	5.0	a	2.5	2.5	a	22.5	100.0	100.0	100.0	a	a
	36	5.0	a	2.5	2.5	a	42.5	100.0	100.0	100.0	a	a
	48	5.0	a	2.5	5.0	a	50.0	100.0	100.0	100.0	a	a
	60	5.0	a	2.5	5.0	a	50.0	100.0	100.0	100.0	a	a
	72	7.5	a	2.5	5.0	a	70.0	100.0	100.0	100.0	a	a
	84	7.5	a	2.5	5.0	a	70.0	100.0	100.0	100.0	a	a
	96	7.5	a	2.5	5.0	a	72.5	100.0	100.0	100.0	a	a
Estuarine fingerlings	6	0.0	a	0.0	0.0	0.0	10.0	a	70.0	95.0	100.0	100.0
	12	10.0	a	0.0	0.0	0.0	35.0	a	90.0	100.0	100.0	100.0
	24	15.0	a	0.0	0.0	60.0	55.0	a	100.0	100.0	100.0	100.0
	48	15.0	a	0.0	0.0	60.0	70.0	a	100.0	100.0	100.0	100.0
	60	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0
	72	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0
	96	15.0	a	0.0	0.0	60.0	75.0	a	100.0	100.0	100.0	100.0

[a]Data were not taken at these concentrations.

methods of Finney (1971) using the probit procedures of SAS (Statistical Analysis System) (SAS Institute 1979) to compare previously published chlorine data with our ozone data. Predictive mortality models for each life stage and water type were derived using the stepwise procedures of SAS (SAS Institute 1979). The following linear model was used in the procedures:

$$\text{Arcsine } [(r/n)^{0.5}] = A + B \text{ concn.} + C \text{ time} + D \text{ dose} + E \text{ concn.}^2 + F \text{ time}^2 + G \text{ dose} \times \text{time} + H \text{ dose} \times \text{concn.} + I \text{ Log (concn.)} + J \text{ Log (time)} + E$$

where

concn. = concentration of OPO (mg/L),
time = exposure time (h),
dose = concentration × time,
r = response in a given experiment,
n = sample size in a given experiment,
$A-J$ = coefficients estimated by least squares procedures.

The important prediction variables were selected by the stepwise procedure and are presented along with their estimated coefficients in the equations in Table 3. Analysis of variance (ANOVA) (Table 4) was used to compare the models between eggs in each type of water (Sokal and Rohlf 1969).

TABLE 3. Predictive percent mortality equations and respective ANOVA data for each life stage. Abbreviations used in the equations are p = percent mortality; c = concentration of OPO (mg/L), and t = exposure time (h). The natural logarithm was used in these equations.

Source	df	Sum of squares	Mean square	F value	Significance
(1) Estuarine eggs: $p = [\sin(0.20 + 13.15c^2)]^2$					
Regression	1	0.08	0.08	2.43	0.13
Error	28	0.90	0.03		
Total	29	0.97			
(2) Freshwater eggs: $p = [\sin(0.22 - 102.73c + 116.66 \log(c + 1))]^2$					
Regression	2	3.19	1.60	80.42	0.0001
Error	27	0.54	0.02		
Total	29	3.73			
(3) Larvae: $p = [\sin(0.005 + 1263.60c - 502.50c^2 + 0.56 \log(t) - 1267.38 \log(c + 1))]^2$					
Regression	4	24.40	6.10	210.32	0.0001
Error	58	1.76	0.03		
Total	62	26.15			
(4) Fingerlings: $p = [\sin(-0.36 - 7.50c^2 + 0.12 \log(t) + 8.33 \log(c + 1))]^2$					
Regression	3	19.87	6.62	110.28	0.0001
Error	50	3.00	0.06		
Total	53	22.88			

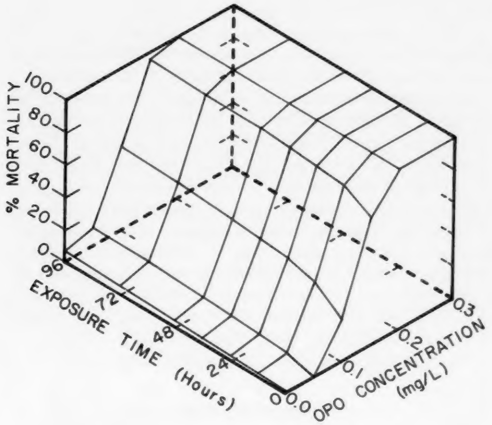

FIG. 1. Three-dimensional response surface of percent mortality for striped bass larvae exposed to OPO concentrations at various exposure times for periods up to 96 h.

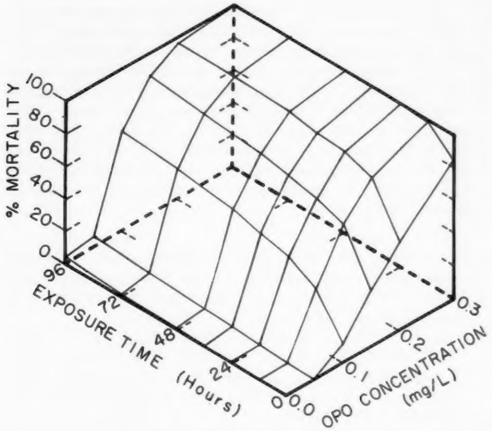

FIG. 2. Three-dimensional response surface of percent mortality for striped bass fingerlings exposed to OPO concentrations at various exposure times for periods up to 96 h.

Results and Discussion

EGGS

Predictive percent mortality models for estuarine and freshwater striped bass eggs are shown in Table 3. In both equations OPO concentration was an important factor contributing to mortality; exposure time was not significant. Eggs tested in estuarine water had a 12- to 30-h LC50 value of 0.21 mg/L OPO when calcualted by predictive equation (1) in Table 3. Eggs were found to be significantly ($P < 0.001$) more sensitive after 12- and 30-h exposures in freshwater than in estuarine water (Table 4). An LC50 value of 0.06 mg/L OPO (95% fiducial limits, 0.03-0.12) was found at both exposure times in freshwater using the probit procedure. A similar LC50 of 0.05 mg/L OPO was calculated from equation (2) in Table 3. The difference in toxicity of ozone in freshwater and estuarine water is probably caused by different residuals that remain after ozonation. Ozone reacts quickly in estuarine water to form several oxidant species (e.g. bromo compounds) which appear to be less toxic than ozone in freshwater which tends to remain longer in the more reactive molecular phase (Kosak-Channing and Helz 1979).

TABLE 4. A comparison of the sensitivity of freshwater and estuarine eggs to OPO using analysis of variance (ANOVA).

Source	df	Sum of squares	Mean square	*F* value	Significance
Water type	4	2.73	0.68	26.53	0.001
Error	52	1.34	0.03		
Total	56	4.07			

Delayed hatching of eggs was observed at 0.05 and 0.10 mg/L OPO in estuarine water. While in freshwater ~70% of the eggs hatched at the expected time (≈48 h after fertilization) at 0.05 and 0.10 mg/L OPO; in estuarine water the hatch was 100% at 0.0 mg/L OPO, 100% at 0.005 mg/L OPO, 73% at 0.01 mg/L OPO, 8% at 0.05 mg/L OPO, and 7% at 0.10 mg/L OPO. It should be noted that in many cases where delayed hatch occurred, embryos were still alive within the chorion. No explanation for this delayed hatching can be given at the present time.

Middaugh et al. (1977) reported 0, 3.5, and 23% hatch for estuarine striped bass eggs continuously exposed for ~40 h to 0.21, 0.07, and 0.01 mg/L TRC, respectively. In our investigation we found 7% hatch for eggs in estuarine water exposed to 0.10 mg/L OPO for approximately the same period of time. It appears in each study that TRC and OPO have a similar effect on percent hatch.

Previous investigators have reported 24- and 48-h LC50's of 0.36 and 0.20−0.22 mg/L TRC, respectively, for striped bass eggs in estuarine water (Morgan and Prince 1977). The 12- to 30-h LC50 value of 0.21 mg/L OPO found in our study is lower than the 24-h LC50 value of Morgan and Prince (1977) but is similar to their 48-h LC50. Morgan and Prince (1978) did find that TRC concentrations of 0.05 mg/L had no significant effect on the percent hatch for this species; however, percent hatch for eggs in estuarine water was affected at 0.05 mg/L OPO in this study.

Burton et al. (1979a, b) conducted chlorine (0.00, 0.15, and 0.30 mg/L TRC), ΔT (2, 6, and 10°C), and exposure duration (0.08, 2, and 4 h) interaction studies with striped bass eggs in estuarine water. These investigators found that TRC and exposure duration were predominant factors contributing to mortality in the test organisms. In a univariate chlorine study, Hall et al. (1981a) found less than 6% mortality for striped bass eggs in estuarine water exposed to TRC concentrations of 0.06 mg/L for 12 h and 100% mortality for 36-h exposures at the same concentration. Exposure time was not found to be an important variable in the present study for eggs in either estuarine or freshwater (see equations (1) and (2) in Table 3). The explanation for the differences in effect of exposure time is not clear; however, it may be related to different oxidant species or water quality conditions present during the studies.

Comparisons of data collected in this study with previously published data indicate that the effects of ozone on estuarine striped bass eggs is similar to that of chlorine. Freshwater chlorine toxicity data on eggs were not available; therefore, comparisons with ozone in this type of water were not possible. The higher sensitivity found for eggs in freshwater suggests that ozone could have a more pronounced effect if discharged in fresh industrial or municipal wastewaters located near a striped bass spawning area.

LARVAE AND FINGERLINGS

Predictive percent mortality equations and response surfaces for striped bass larvae and fingerlings in estuarine water are shown in Table 3 and Fig. 1 and 2, respectively. Ozone-produced oxidant concentration and exposure time contributed to mortality in both life stages. The LC50 values for the larvae ranged from 0.15 mg/L OPO (95% fiducial limits, undefined) at 6 h to 0.08 mg/L OPO (95% fiducial limits, 0.05−0.11) at 96 h. LC50's for fingerlings were 0.20 mg/L OPO (95% fiducial limits 0.13−0.27) at 6 h and 0.08 mg/L OPO (95% fiducial limits, 0.05−0.13) at 96 h. Mortality increased over time but tended to stabilize at the longer exposure periods for both life stages.

Middaugh et al. (1977) reported 48-h LC50 values of 0.04 and 0.07 mg/L TRC for 2- and 12-d-old striped bass larvae. In our investigation we found a 48-h LC50 of 0.09 mg/L OPO (95% fiducial limits, 0.07−0.12) for this developmental stage. Comparison of data from these two studies shows that larvae exhibit a similar response to both biocides.

Morgan and Prince (1977) reported 24-h LC50's of 0.20 and 0.19 mg/L TRC for 24- and 70-h-old estuarine striped bass larvae, respectively. The 24-h LC50 for larvae in our study was 0.11 mg/L OPO (95% fiducial limits, 0.04−0.20). Since our 95% fiducial limits overlapped the LC50's found by Morgan and Prince, it appears that the effects from each oxidant are similar.

Chlorine (0.00, 0.15, and 0.30 mg/L TRC), ΔT (2, 6, and 10°C), and exposure duration (0.08, 2, and 4 h) interaction studies have been conducted by Burton et al. (1979a, b) with 24-h-old estuarine striped bass prolarvae. Total residual chlorine, ΔT, and exposure time were all found to be important factors causing mortality to this life stage. Precise comparisons between the Burton et al. studies and the present investigation are difficult because of different testing procedures. However, Burton et al. found 100% mortality after 4 h of exposure at a concentration of 0.15 mg/L TRC and a ΔT of 0°C while in the present study we found a 6-h LC50 of ~0.15 mg/L OPO (95% fiducial limits, undefined). The difference in mortality response for the larvae in each study was probably due to differences in the ages of test organisms.

Previous investigations in our laboratory have shown that ~75% mortality occurred with estuarine striped bass larvae (7−9 mm) subjected to 0.06 mg/L TRC for 96 h (Hall et al. 1981a). In the present study we found a 96-h LC50 of 0.08 mg/L OPO (95% fiducial limits, 0.05−0.11) for striped bass larvae. A comparison between these studies suggess that larvae may be slightly more sensitive to TRC than OPO; however, this difference does not appear to be very large.

A comparison between data collected in this study and the published literature indicates that the toxicities of ozone and chlorine are generally similar for striped bass larvae in estuarine water. A comparison for fingerlings was not possible due to lack of chlorine data. In previous studies, Helz et al. (1978) reported similar bromoform yields from ozone and chlorine in estuarine water indicating that the reaction pathways and mechanisms may be similar with these two biocides. In view of the results of this investigation, we recommend that further site-specific studies be conducted with OPO and TRC in estuarine water of the same quality before decisions can be made concerning the possible use of ozone in estuarine industrial or municipal wastwaters.

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Advanced Photoperiod and Water Temperature Effects on Gill Na^+–K^+ Adenosine Triphosphatase Activity and Migration of Juvenile Steelhead (*Salmo gairdneri*)

W. S. ZAUGG[1]

National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Coastal Zone and Estuarine Studies Division, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

ZAUGG, W. S. 1981. Advanced photoperiod and water temperature effects on gill Na^+–K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 38: 758–764.

Under raceway conditions, an advanced photoperiod schedule caused migratory movements and elevation in gill Na^+–K^+ adenosine triphosphatase activity (Na^+–K^+ ATPase) to occur about 1 mo earlier than normal in yearling summer steelhead (*Salmo gairdneri*) from Dworshak National Fish Hatchery (Idaho). Exposure of migrants to 13°C for 20 d resulted in serious impairment of continued migratory behavior and a reduction of gill Na^+–K^+ ATPase activity. Migrants outnumbered nonmigrants at fork lengths of 16 cm and longer. It is proposed that the potentially detrimental effects of warming river temperatures during the normal migratory season and delayed migration caused by dams and impoundments might be partially overcome by inducing early smolt transformation and migration with the use of advanced photoperiods.

Key words: ATPase, steelhead, migration, temperature, photoperiod, smolts

ZAUGG, W. S. 1981. Advanced photoperiod and water temperature effects on gill Na^+–K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 38. 758–764.

Chez des truites steelhead (*Salmo gairdneri*) d'été d'un an maintenues dans des canaux à l'établissement de pisciculture national de Dworshak (idaho), une régime de photopériodes avancées résulte en des mouvements migratoires et en une élévation de l'activité de la Na^+–K^+ adénosine triphosphatase (Na^+–K^+ ATPase) branchiale à peu près 1 mois avant la date normale. Une exposition à 13°C pendant 20 jours des poissons qui émigrent dérange notablement le comportement migratoire et cause une diminution de la Na^+–K^+ ATPase branchiale. À des longueurs à la fourche de 16 cm et plus, les poissons qui émigrent dépassent en nombre les non migrateurs. Nous sommes d'avis que les effets potentiellement nuisibles de l'élévation de la température dans les rivières durant les saisons normales de migration et le retard à la migration dû aux barrages et à la retenue des eaux peuvent être partiellement compensés en provoquant une transformation et une migration hâtives des smolts par des photopériodes avancées.

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HATCHERY programs for rearing anadromous salmonids can be successful only if the juvenile fish are able to complete seaward migration and fully acclimate to the seawater environment. However, this is not always possible for steelhead (*Salmo gairdneri*) from regions of the upper Columbia and Snake River drainages. In their attempt to reach the ocean, these fish must pass through hundreds of kilometres of a nearly continuous chain of dam reservoirs at rates greatly reduced from those in free-flowing streams (Raymond 1968, 1969). At these reduced rates of travel, their ability to reach the estuary prior to reverting to a nonmigratory stage, which normally occurs in late June (Conte and Wagner 1965; Zaugg et al. 1972), is placed in jeopardy. Raymond et al. (1974, 1975) observed that more steelhead remained in Snake and Columbia River reservoirs and did not complete their seaward migration in the low-flow year of 1973 than in 1974, a year of relatively high spring flows. Likewise, Sims et al. (1978) reported that large numbers of steelhead did not migrate from reservoirs in 1977, another record low-flow year.

Rising river temperatures, especially manifested in years of low water flow, impose additional threats to successful seaward migration. Under laboratory conditions temperatures of 12.7 and 15°C inhibited the development of elevated gill Na^+–K^+ ATPase activity and increased mortality due to salt-

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[1] Current address: National Marine Fisheries Service, Cook Field Station, Cook, WA 98605, USA.

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water exposure of steelhead (Zaugg et al. 1972; Adams et al. 1973, 1975). Zaugg and Wagner (1973) found that elevated water temperature not only decreased gill Na$^+$–K$^+$ ATPase enzyme activity but also inhibited downstream migration of winter steelhead.

Many investigators feel that summer run steelhead, which migrate great distances, may not respond physiologically to photoperiod changes and temperature differences in the same manner as the coastal, or winter run, species which migrate only short distances. Experiments reported in this paper demonstrate that summer run steelhead do respond in a similar way to these environmental stimuli and that temperature conditions presently existing in the Columbia River pose a real threat to successful migration of smolts from distant upriver locations.

Materials and Methods

Yearling juvenile steelhead were brought to the Willard National Fish Hatchery, Washington, from Dworshak National Fish Hatchery, Idaho, on January 7, 1976. On January 8, two groups of ~400 fish were placed in the upper pool of separate 3 × 25 m production raceways which had been modified for testing migration. This modification consisted of placing one dam about 7.7 m from the upper end of the raceway and a second lower dam at the midpoint of the raceway (about 4.3 m from the first). A screen was placed across the first dam for 1 mo to prevent escapement of fish until they had accustomed themselves to the new surroundings. A plywood covering was placed over the lower portion of the first pool (just above the first dam) to provide cover for the fish. Water below the second dam was kept at a low level (about 15 cm) to prevent return of fish to the upper pools once migration over both dams had been accomplished. Migrating fish were netted from the lower pond and, until April 8, were returned to the upper pond.

One of the two modified raceways was equipped with electric lighting to extend daily photoperiods during the evening hours. Light from this raceway had no influence on the natural lighting of the control group. Lights consisted of three equidistantly spaced clusters of three 300-W outdoor flood lamps 1.7 m above the water surface of the upper pond. The lights were turned on at 15:00 each day and were left on each evening according to the following schedule:

Dates (1976)	Lights off
Jan. 9–Jan. 19	21:00
Jan. 20–Jan. 29	21:15
Jan. 30–Feb. 8	21:30
Feb. 9–Feb. 17	21:45
Feb. 18–Feb. 26	22:00
Feb. 27–Mar. 7	22:15
Mar. 8–Mar. 17	22:30
Mar. 18–Mar. 28	22:45
Mar. 29–Apr. 7	23:00
Apr. 8–Apr. 19	23:15

On April 19, after good migration had developed in fish under the advanced photoperiod schedule (AP), lights were turned off. From April 8 to May 5 all migrants that received AP and natural light (NL) were captured from their respective last pools in the raceways and, with the exception of a few abnormally small fish, were marked with finclips and placed in Fiberglas tanks, indoors, under normal photoperiod conditions, and in water at 6°C. The NL migrants were divided into two groups. Individual fish in one group were marked with an adipose and a right ventral finclip and fish in the other group were marked with an adipose and left ventral clip. The AP migrants were also divided, one group receiving right ventral and the other left ventral clips. All fish having the same ventral clip, both NL and AP, were placed in a common tank so that on May 5, one tank contained 45 fish with adipose–left ventral clips (NL) and 89 fish with left ventral clips only (AP) while another tank contained 44 fish with adipose–right ventral clips (NL) and 92 fish with right ventral clips only (AP).

On May 5, water temperature in the tank containing fish with left ventral clips was raised to 13°C. Water in the other tank, containing fish with right ventral clips, remained at 6°C. After 20 d under these conditions, both groups of fish were placed together in the upper pool of a raceway modified for migration testing. A screen preventing downstream movement was left in place on the upper dam for 2 d. Fish migrating to the lower pool were then collected, measured, and tested for gill Na$^+$–K$^+$ ATPase activities until June 16, at which time all migration had ceased. Those fish remaining in the upper pond were retrieved and measured and several were tested for Na$^+$–K$^+$ ATPase activity. Water temperatures in the raceways had ranged from 4 to 7°C during these studies.

Throughout the study Na$^+$–K$^+$ ATPase activities were determined on microsomal fractions of gill homogenates from individual fish or from groups by combining approximately equal quantities of gill filaments from the individual fish. Procedures were similar to those used and reported previously (Zaugg and McLain 1970), differing primarily by a 50% reduction in centrifuging times. Activities are reported as micromoles ATP hydrolyzed per milligram protein per hour.

Results

Elevation of gill Na$^+$–K$^+$ ATPase activity was first detected on May 7 in migrants receiving NL collected from the lower pool of the modified raceway (Table 1) and on April 2 in migrants under AP (Table 2). Upper ponds (nonmigrants, Tables 1 and 2), from which several samples were taken during the experiment, were not sampled at times of peak migration in order to avoid possible downstream movement due to fright.

From February 23 to March 22 straying over the dams was about the same for both NL and AP groups and averaged less than one fish per day. Beginning on March 22, there was much more downstream movement in the AP raceway, but until April 6 all fish that migrated (except those tested for ATPase activity) were returned to the upper pools (102 AP and 12 NL fish). On April 7 and thereafter, all migrants were removed from the system. Figure 1 records the daily movement of fish after April 6 and therefore indicates only about a 2-wk difference in timing between AP and NL migrations. In reality, however, the type of migratory activity observed in AP fish beginning about March 22 was not observed in NL fish until early May, thus making a difference of more than

TABLE. 1. Gill Na^+–K^+ ATPase activities and fork lengths of yearling steelhead under natural light. Asterisks indicate values significantly greater than those prior to May 7 (*$P < 0.05$, *t*-test).

Group and date	Mean ATPase activities[a]	Standard error	Mean length (cm)	Range of lengths (cm)	No. of fish
Migrants (lower pond)					
Feb. 23	20.5	1.5	14.5	13.6–17.1	6
Mar. 10	15.7	0.5	14.8	14.1–15.3	5
Apr. 6	21.0	0.8	16.5	16.1–16.9	3
May 7	32.0*	4.0	16.5	16.2–16.7	4
May 14	32.0*	—	16.2	16.0–16.4	3
May 24	37.3*	4.4	16.3	13.5–19.3	6
June 3	37.1*	2.9	15.6	15.1–16.5	7
June 7	33.3*	1.7	17.1	15.3–18.6	9
June 18[b]	39.3*	0.8	18.7	17.5–19.6	8
Nonmigrants (upper pond)					
Feb. 10	19.0	2.0	13.7	11.9–13.9	6
Mar. 23	15.5	0.5	17.0	15.8–18.4	12
Apr. 19	20.8	0.7	16.9	15.2–18.9	11
June 16	19.8	1.5	16.5	13.7–19.1	12

[a] Micromoles ATP hydrolyzed per milligram protein per hour.
[b] Migrated from May 5 to May 25, 1976 and held inside at 6°C until this date.

TABLE. 2. Gill Na^+–K^+ ATPase activities and fork lengths of yearling steelhead on an advanced photoperiod schedule. Asterisks indicate values significantly greater than those prior to April 2 (*$P = 0.1$, **$P < 0.05$).

Group and date	Mean ATPase activities[a]	Standard error	Mean length (cm)	Range of lengths (cm)	No. of fish
Migrants (lower pond)					
Feb. 10	17.7	0.3	14.7	13.2–16.9	9
Feb. 13	20.5	0.5	14.3	12.9–16.3	6
Feb. 26	18.5	1.1	15.3	13.8–16.5	6
Mar. 10	18.4	1.7	16.1	14.4–18.0	5
Mar. 24	21.5	1.5	16.8	16.7–16.9	2
Apr. 2	27.8*	1.9	16.1	12.5–18.3	5
Apr. 6	26.7*	1.1	17.2	15.7–18.5	7
Apr. 12	23.7	1.6	16.4	15.2–18.0	3
Apr. 16	25.9*	1.2	15.9	14.7–17.1	8
May 7	37.3**	1.0	16.6	14.3–18.7	9
May 17	32.5**	6.1	17.4	15.3–19.9	4
June 18[b]	28.6**	2.9	19.3	18.5–20.4	7
Nonmigrants (upper pond)					
Feb. 10	14.3	1.9	15.0	13.3–16.5	9
Mar. 23	18.8	2.1	16.2	15.2–17.7	10
June 3	26.0	2.1	16.7	14.2–19.8	13
June 15	21.0	2.6	18.0	15.2–19.6	8

[a] Micromoles ATP hydrolyzed per milligram protein per hour.
[b] Migrated from May 5 to May 25, 1976 and held inside at 6°C until this date.

4 wk between the two groups in the initiation of strong migratory behavior.

Steelhead exposed to 13°C water for 20 d had a reduced tendency to migrate (Fig. 2 and 3). The effect was greater on AP fish than on NL fish and also resulted in depressed gill Na^+–K^+ ATPase activities (Table 3).

Comparing numbers of migrants with numbers of nonmigrants in fork length increments of 0.5 cm shows that migrants began to outnumber nonmigrants noticeably at 16.0–16.5 cm for both NL (Fig. 4) and AP (Fig. 5) fish. Nevertheless, several fish (22) in the fork length range of 12.5–15.7 cm migrated and had elevated gill Na^+–K^+ ATPase activities (28 ± 2 (SE)) which compares with seven nonmigrants, 13.9–15.9 cm fork length, with lower ATPase activities (17 ± 2 (SE)). These data suggest a minimum fork length of about 16 cm for a high percentage of migrants.

Discussion and Conclusions

The studies reported here have shown that migratory behavior and elevation in gill Na^+–K^+ ATPase activity in summer run steelhead from Dworshak National Fish Hatchery can be advanced at least 1 mo under conditions similar to those existing in a production facility by advancing photoperiods

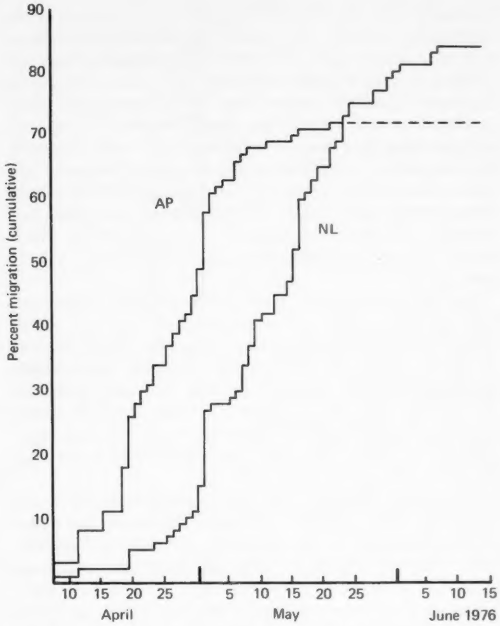

FIG. 1. Migration of yearling steelhead subjected to natural light (NL) or to advanced photoperiods (AP).

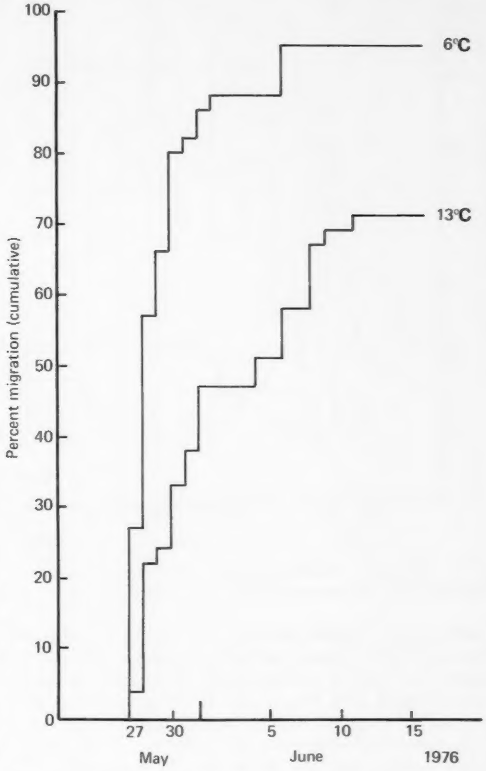

FIG. 2. Migration of yearling steelhead smolts from natural light which had been exposed for 20 d to either 6 or 13°C water.

through the use of electric lighting. Thus, steelhead from the upper river drainages appear to respond to photoperiod adjustment much as winter run steelhead (Wagner 1974; Zaugg and Wagner 1973) with respect to these indicators of smolt transformation (Hoar 1976). Fish receiving advanced photoperiods migrated initially nearly as well as those on natural light (Fig. 1). However, after being held (at 6°C) under natural light conditions from 3 to 6 wk, while other fish were being collected, migration was much less than NL fish (Fig. 2 and 3). Apparently, in addition to an advance in the manifestation of these smolt indicators, the AP group must have experienced an accelerated reversion to a nonmigratory parr, or postsmolt state, an event that normally occurs from early to late June, depending on water temperatures. This reversion may have resulted from response to either an advance in the biological clock or to the abrupt change from an advanced (long) to a normal, shorter photoperiod. What effect this might have on animals liberated into a natural environment after experiencing early development of smolting indicators by advanced photoperiods is unknown. Recent studies (W. S. Zaugg, National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Coastal Zone and Estuarine Studies Division, 2725 Montlake Boulevard East, Seattle, WA 98112, USA, unpublished results) have shown that coho salmon (*Oncorhynchus kisutch*) released in June and July, after having experienced an increase then a decrease in gill Na$^+$–K$^+$ ATPase activity while in the hatchery environment, rapidly migrated seaward with elevating ATPase levels. It is quite possible that, in spite of the abrupt change in photoperiod, steelhead induced to initiate the smolting process early would be less likely to revert prematurely while actively migrating in a natural river than while being confined under the conditions of this experiment. However, this supposition is necessarily subject to experimental verification.

Presently, peak steelhead migration from the upper Columbia and Snake rivers occurs from early to mid-May, with some movement being observed during the latter half of April and into June (Bjornn et al. 1978; Chrisp and Bjornn 1978; Ebel et al. 1975; Raymond et al. 1975). Using migration rates for moderate flows determined by Raymond et al. (1975), Chrisp and Bjornn (1978) calculated the travel time for steelhead from the Pahsimeroi and Lemhi rivers in Idaho to the Columbia River estuary at 57 d. Migrants leaving these streams on the first of May would theoretically arrive at the estuary near the end of June, at a time when normal reversion occurs. Therefore, even in years of moderate water flow many steelhead smolts may not be able to reach the estuary from upper river regions before some loss of smolt condition. Consider, then, the potential loss of smolts in years of low water flow, when the calculated migrating time is 94 d (Chrisp and Bjornn 1978).

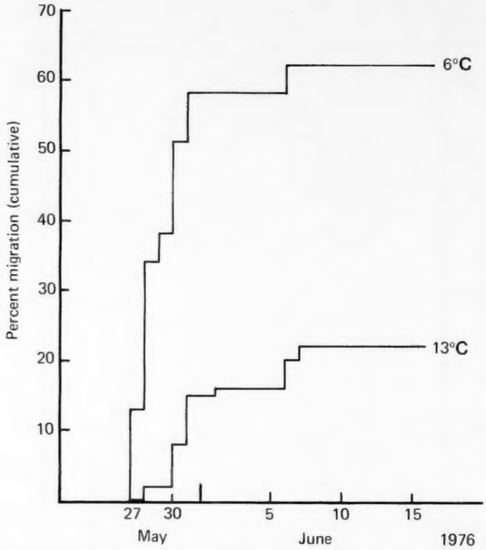

FIG. 3. Migration of yearling steelhead smolts from advanced photoperiods which had been exposed for 20 d to either 6 or 13°C water.

In this study steelhead which had previously migrated to the lower end of the raceway and then were exposed to a 13°C temperature for 20 d showed a reduced tendency to move down a second time (Fig. 2 and 3). Even those fish that migrated had lower gill Na^+–K^+ ATPase activities than control fish which had been held at 6°C (Table 3). Lower enzyme activity indicates that these fish were in the process of reverting and would not be capable of a sustained downstream migration in this state. This conclusion is supported by observations from several studies that all active, seaward, migrating steelhead examined have had elevated gill Na^+–K^+ ATPase levels.

Temperatures of the Columbia River at Bonneville Dam generally reach 12°C by mid-May. However, this can occur much earlier in years of low water flow, for example on May 5 in 1973 and on April 28 in 1977. If seaward migrants are affected by river temperatures above 12°C as are test fish in laboratory situations (Adams et al. 1975), then present river conditions pose a very serious threat. Temperature-induced reversion may have been an important factor in the failure of large numbers of steelhead smolts to complete seaward migration and to hold over in 1973 and again in 1977 in Lower Monumental, Little Goose, Lower Granite, and John Day reservoirs (Raymond et al. 1974; Sims et al. 1978). Such reversions may, and probably do, also occur in years of moderate flow.

An optimum length of 20 cm has been suggested for summer steelhead reared 1 yr in hatcheries (Chrisp and Bjornn 1978). However, the present study shows that fish as small as 16 cm are capable of good smolt development where factors such as pond density, water temperature, and water quality do not interfere with this development. Under the right conditions then, size should not be limiting in the ability of steelhead at Dworshak to undergo early smolt transformation induced by advanced photoperiods.

If steelhead which initiate smolt transformation early as a result of advanced photoperiods (and consequently begin seaward migration up to 1 mo earlier than normal) could successfully complete migration, then the potentially disastrous effects of warming river water might be avoided. Early migrating steelhead would likely reach the estuary at a time corresponding more closely to historic arrivals, which were not delayed by reservoirs and dams, and would be exposed to fewer disease organisms than might be encountered later in warmer water.

It is proposed that early migrating steelhead should be able to overcome in some measure the adverse conditions of dam-delayed migration and warm river temperatures, and that further studies should be undertaken to explore the potential for success of such an approach.

Acknowledgments

I express sincere appreciation to Lynn McLain and Bonnie Ternahan for their technical assistance and to personnel at Dworshak

TABLE. 3. Gill Na^+–K^+ ATPase activities in yearling steelhead under natural light (NL) and advanced photoperiods (AP) exposed to 6 and 13°C water. Enzyme activities determined on May 28–June 7 were on fish retrieved from the lower ponds whereas fish used on June 17 were from the upper ponds.

Date	Light	Temperature (°C)	No. of fish	Mean fork length (cm)	Length range (cm)	Mean ATPase[a]	Standard error
May 28–June 7	NL	6	8	18.2	16.1–20.6	34.8	1.3
	NL	13	10	18.4	17.0–20.1	19.0	1.7
	AP	6	10	17.5	15.8–19.0	34.8	3.1
	AP	13	6	17.5	16.0–20.0	19.7	1.4
June 17	NL	6	1	19.5	—	37.0	—
	NL	13	6	17.7	16.7–19.0	19.7	0.6
	AP	6	6	17.2	15.6–19.3	21.7	2.7
	AP	13	6	18.1	16.8–18.8	19.0	1.1

[a] Na^+–K^+ ATPase activity expressed as micromoles ATP hydrolyzed per milligram protein per hour. All differences in activities between temperature treatments were significant ($P < 0.05$) except for AP on June 13 which showed no significant difference.

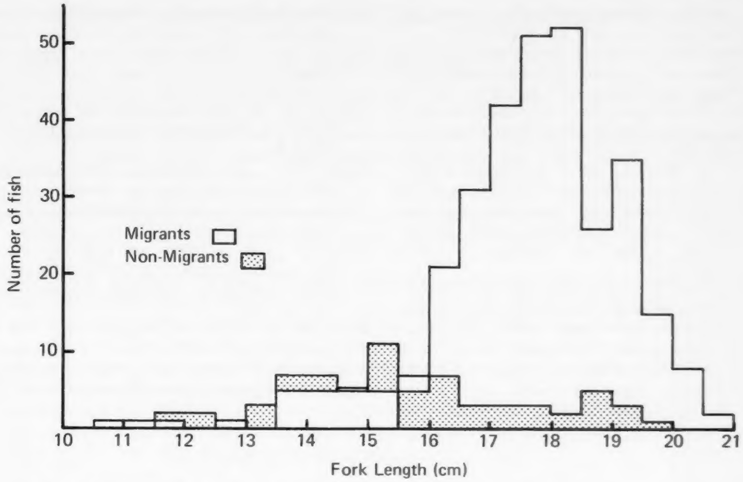

FIG. 4. Relationship of fork length to migration of steelhead under natural light.

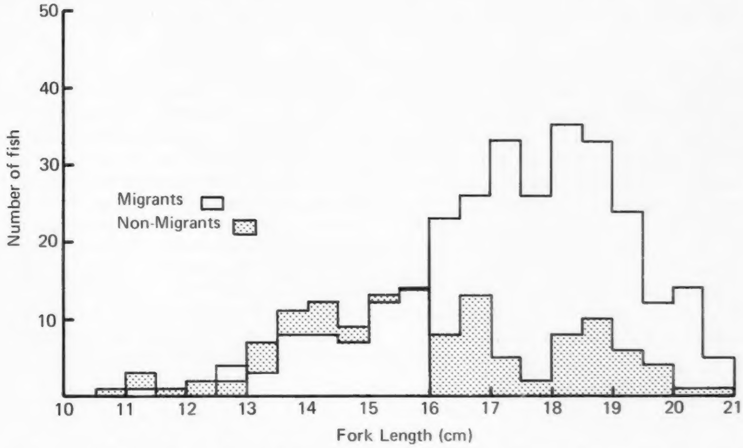

FIG. 5. Relationship of fork length to migration of steelhead under advanced photoperiods.

National Fish Hatchery for their cooperation in these studies. This study was funded by the United States Fish and Wildlife Service, Division of Fish Hatcheries.

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The First Diets of Postemergent Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon (*Salmo salar*) Alevins in a Quebec River[1]

D. DUDLEY WILLIAMS

Division of Life Sciences, Scarborough College, University of Toronto, West Hill, Ont. M1C 1A4

WILLIAMS, D. D. 1981. The first diets of postemergent brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) alevins in a Quebec river. Can. J. Fish. Aquat. Sci. 38: 765–771.

In the Matamek River, Quebec, brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) coexist. The trout alevins emerge from the redds in early June and the salmon ~1 mo later. The first diet of the trout consists primarily of small- to medium-sized invertebrates passing in the water column. Among the most common items are blackfly larvae and zooplankton derived from an upstream lake. The alevins also feed at the water surface and take considerable numbers of emerging adults of orthocladine chironomids. There appears to be diel variation in the importance of various dietary species. The salmon alevins hold station in faster water and tend to feed more from the substrate on medium- to large-sized invertebrates. Their first meals include large stonefly nymphs, chironomid pupae, and larval dytiscid beetles. The salmon also feed extensively on adult chironomids and caddisflies as they emerge at the water surface. All of these items are important in the diet of the older brook trout at this time. The concept of food availability is discussed.

Key words: brook trout, Atlantic salmon, alevins, feeding behavior, invertebrates, drift

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Dans la rivière Matamek, au Québec, coexistent l'omble de fontaine (*Salvelinus fontinalis*) et le saumon de l'Atlantique (*Salmo salar*). Les alevins de truites éclosent au début de juin, ceux de saumons ~1 mois plus tard. Les premiers se nourrissent d'abord d'invertébrés petits à moyens, surtout, qui passent dans la colonne d'eau. Leur régime est alors surtout constitué de mouches noires et de zooplancton provenant d'un lac en amont. Les alevins se nourrissent aussi à la surface de l'eau et dévorent un nombre considérable d'imagos de chironomidés (Orthocladinae) au moment de leur sortie. Il semble y avoir une variation diurne de la contribution des diverses espèces au régime. Les alevins de saumon se cantonnent dans les eaux les plus rapides et se nourrissent plutôt sur le substrat d'invertébrés gros ou moyens. Leurs premiers repas comprennent des grosses nymphes de perles, des chrysalides de chironomidés et des larves de tigres d'eau. Les saumons se nourrissent aussi beaucoup d'imagos de chironomidés et de phryganes au moment de leur émergence. Tous ces insectes constituent une part importante du régime des alevins de truites, plus âgés, à ce moment. L'auteur discute aussi des disponibilités de nourriture.

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IN eastern Canada, the eggs of both the brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) are laid in the fall. These eggs incubate in the substrate over the winter with development time depending upon factors such as temperature and dissolved oxygen. After hatching, the alevins remain within the substrate until their yolk sacs have been absorbed (Scott and Crossman 1973). Finally, they emerge into the water column and begin to feed. In the Matamek River, Quebec, brook trout generally emerge in early June whereas salmon emerge ~4 wk later. Although a considerable number of feeding studies on these two species exist (e.g. Ricker 1932; Allen 1940; Lillehammer 1973a), some of them on the Matamek River stocks themselves (e.g. Gibson and Galbraith 1975), these invariably deal with fishes that are older than 1 mo. To date, apart, from preliminary notes made by White (1930, 1937), no detailed study of the first diets of these fish species exists.

The Study Area

The Matamek River is a low-productivity, brown-water river which flows into the Gulf of St. Lawrence on its northern shore ~20 km east of Sept Iles, Quebec (50°18′N, 65°57′W).

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[1] This is Contribution No. 53 from the Woods Hole Oceanographic Institution's Matamek Research Station.

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The river drains the large Matamek Lake and has a watershed of over 600 km^2 comprising Precambrian bedrock covered in places by glacial and marine sediments and thin acid soils. It is densely forested (Boreal Forest Region), with black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), white birch (*Betula papyrifera*), and alder (*Alnus* spp.) predominating (Williams and Williams 1979). The river is ~10 km long and drops 120 m in elevation over five large waterfalls. It has a maximum width of about 60 m and depth in summer varies from a few centimetres near gravel bars to several metres in pools. The substrate varies from bedrock and large cobbles at the fifth (uppermost) falls, through gravel, to sand and accumulated allochthonous organic debris in the pools. For a more detailed description of the river see Gibson (1973).

Materials and Methods

Brook trout were collected in the 1st wk of June 1979 as they began to leave the redds. Sampling was begun before the predicted time of emergence so that the first alevins caught were unmistakably newly emerged. Most collections were made with a dip net on the east and west sides of a small island below the third falls, just downstream of a major spawning site. Approximately 20 animals were taken each time and care was taken not to stir up the substrate which might have affected the diets of animals subsequently sampled. Three samples were taken over a 24-h period (07:30, 14:30, and 22:00) in order to establish any diel change in feeding preference. Two drift nets of 53-μm mesh were set on either side of the island, one in shallow water where the fishes occurred and one in deeper water ~2 m out from the banks. These nets were emptied and reset at the same time as the fishes were sampled. At the end of the 24-h series, benthic samples were taken, using a kick-sampling technique, from the substrate in the area where the fishes were holding station. All samples, both vertebrate and invertebrate, were preserved in 10% Formalin solution.

At the 14:30 sampling time, an additional 80 alevins were taken for a study of digestion rate; 20 of these were preserved immediately and represented a reference group for comparison. The remainder were taken back to the laboratory and held in tanks of vigorously aerated river water at river temperature (13.3°C). At 6-h intervals thereafter, 12 alevins were killed and preserved so that the amount of material remaining in the guts could be compared.

Twenty alevins were collected also from below the fifth falls for evidence of any longitudinal variation in diet within the river. Whenever samples of brook trout were taken, prior underwater observations were made of their feeding behavior. No salmon occur at or above the fifth falls.

Gut analyses consisted of removing the entire stomach from each fish, making an estimate of fullness to the nearest one-quarter, cutting the gut open, and identifying, counting, and estimating the percentage composition of each food type present. Conventional methods of gut analyses (e.g. Sosiak et al. 1979) were impracticable due to the very small size of the fishes involved. Most of the material recovered was intact and some food items could be identified to species. However, wing and other taxonomic characters of adult chironomids were invariably partly digested, thus preventing specific identifications of these important components.

In July 1979, unusually high river levels either severely depleted the stocks of postemergent salmon alevins or made them less accessible to collection. Consequently, gut analyses were done on newly emerged specimens collected with a dip net below the third falls on July 11, 1976. These guts are compared with those of coexisting 4-wk-old brook trout collected in the same area at this time. Reference to a series of benthic samples, taken by various workers, from the Matamek River ruled out any change in the species composition of the fauna over the period 1976−79.

Results

Upon emerging, brook trout alevins at the third falls are swept downstream and collect in areas of reduced current along the margins of the island. Water depth on the west side of the island in early June varied from 40 to 65 cm and the current within 1 m of the island was about 10 cm/s although large eddy currents also occurred. Corresponding values on the east side were 17−20 cm/s for current and 25−54 cm for depth. Alevins found off the west bank were generally under an overhanging section of bank whereas those off the east bank were under a dense canopy of alder branches. Daytime, underwater observations of the feeding behavior revealed that the fishes were specifically in midwater just on the edge of the shadows cast by the bank overhang and the riparian vegetation. Each fish tended to hold a set territory but would leave it to rise for anything, moving or stationary, which appeared on the water surface. Any passing object in the water column was similarly investigated and eaten if suitable; frequently the object was abandoned purely on visual grounds but whether this decision was made on the grounds of size, shape, or color could not be determined. Alevins would swim down to objects moving close to, but not on, the bottom and generally returned to their stations after each exploratory sojourn. Only when material was stirred up by the observer did the fishes feed on the bottom. They seemed little bothered by the presence of the observer but some intraspecific aggression was evident.

Table 1 shows the diel variation in the stomach contents of brook trout that were no more than 1 or 2 d postemergence. These fish averaged 25.1 mm in length (range 20.0−27.0 mm) and had a mean weight of 87 mg (range 70−120 mg). Many had remnants of yolk in the gut. The total number of each food item found in the stomachs of each collection of fish is given, as is the frequency of occurrence of each item (expressed as a percentage) in all the stomachs of a given collection. Finally, a volumetric estimate (expressed as a percentage) of the contribution of each item to an average stomach is made. The latter two parameters were both deemed necessary as certain dietary items showed differences in importance depending on whether presence in the stomachs was measured in terms of numbers or volume occupied. For example, in the 07:30 collection, *Bosmina longirostris* occurred in most stomachs (73.3%) but only constituted, on average, 8.7% of the volume of each stomach.

The greatest proportion of the stomachs of alevins collected at 07:30 was taken up by larvae of *Prosimulium fuscum* (28.3% by volume). These varied in size from middle to late instar and some stomachs contained as many as 17 larvae. The next most important item in the guts was emerging adults of

TABLE 1. Diel variation in the stomach contents of postemergent brook trout collected at the third falls island.

	S. fontinalis (07:30) (n = 15)			*S. fontinalis* (14:30) (n = 20)			*S. fontinalis* (22:00) (n = 15)		
Food item	Total no. found	Frequency in stomachs (%)	Avg. % vol. per stomach	Total no. found	Frequency in stomachs (%)	Avg. % vol. per stomach	Total no. found	Frequency in stomachs (%)	Avg. % vol. per stomach
Nematoda	1	6.7	1.0	1	5.0	0.5	2	6.7	1.3
Oligochaeta	1	6.7	1.3	2	10.0	0.3			
Cladocera									
Bosmina longirostris	78	73.3	8.7	224	80.0	15.8	27	53.3	5.5
Copepoda									
Diaptomus minutus and *Cyclops scutifer*	103	86.7	13.4	175	85.0	15.3	28	60.0	3.7
Acari				1	5.0	0.3			
Collembola	3	13.3	3.7						
Thysaneura				7	25.0	2.3			
Plecoptera	1	6.7	0.1						
Ephemeroptera							1	6.7	0.1
Hemiptera: Gerridae	1	6.7	0.7	1	5.0	1.3			
Trichoptera:									
Limnephilidae	1	6.7	0.1						
Chimarra sp.	20	46.7	4.1	38	75.0	6.8			
Diptera:									
Ceratopogonidae							3	13.3	0.8
Empididae	4	20.0	1.5	1	5.0	0.3			
Prosimulium fuscum	63	80.0	28.3	56	80.0	24.3	76	93.3	36.7
Chironomidae									
Ablabesmyia sp.							9	33.3	3.7
Pentaneurini (unidentifiable)				7	20.0	1.5	4	13.3	4.7
Eukiefferiella sp.	16	60.0	3.6	18	45.0	3.3	42	73.3	7.7
Psectrocladius sp.				13	45.0	2.3	5	26.7	4.1
Heterotrissocladius sp.							2	13.3	2.7
Cricotopus sp.	12	60.0	3.4	16	35.0	4.0			
Corynoneura sp.	3	13.3	0.7	1	5.0	0.3			
Synorthocladius sp.	10	40.0	2.3	7	25.0	1.5			
Polypedilum sp.	1	6.7	0.3				1	6.7	0.1
Phaenopsectra sp.							1	6.7	0.1
Micropsectra sp.	6	26.7	1.5	8	25.0	1.8	17	73.3	6.7
Emerging adults (unidentifiable)	27	60.0	17.7	10	40.0	6.8	13	66.7	18.6
Pupae (unidentifiable)	5	20.0	4.7	10	30.0	7.8			
Unidentifiable material		2.9			3.5				3.5
Total number of taxa		19			19			16	

orthocladine chironomids (17.7% by volume) followed by *Diaptomus minutus* and *Cyclops scutifer* (13.4% combined). These four taxa accounted for almost 60% of the total volume of food in an average stomach. Other significant items were *B. longirostris* and orthocladine pupae (8.7% and 4.7%, respectively). A total of 19 different taxa was eaten during the early morning hours.

In the 14:30 collection, *P. fuscum* was still the major item eaten (24.3% by volume) but emerging orthoclads had decreased to 6.8%, probably a reflection of their decreased availability at this time of day. *Diaptomus* and *Cyclops* were still important components (15.3%) and the proportion of *Bosmina* had increased to 15.8%, possibly as they were more visible in bright daylight. Orthocladine pupae contributed 7.8%. A few species which were not eaten in the early morning contributed minor amounts, for example, larval Pentaneurini, *Psectrocladius* sp., and terrestrial thrips (Thysaneura). In addition the contribution of first instar net-spinning Trichoptera (*Chimarra* sp.) increased to 6.8%.

There was an increase in the contribution of *P. fuscum* to the stomach contents of the 22:00 collection (to 36.7% by volume); probably this reflected its increased abundance in the drift in the early evening. Emerging adult orthoclads became more important once again (18.6%) while the proportions of copepods and cladocerans decreased significantly. Slight increases in the contributions of certain chironomid larvae (e.g. *Eukiefferiella*, *Ablabesmyia*, and *Micropsectra*) occurred, most likely caused by their times of activity and consequent availability to the trout.

Comparison of the fullness of the stomachs at the different collecting times showed that stomachs of fishes collected at 07:30 ranged from one-quarter full to full, with a fairly even distribution over the range. Stomachs from the 14:30 collection ranged between one-quarter full and full, with 85% being over one-half full, and stomachs from the 22:00 collection were also mostly one-half full or more (67%) and ranged from one-quarter full to distended. The total number of taxa eaten at the different times remained relatively constant.

Table 2 gives an idea of the retention time of food in the stomachs at 13.3°C (river water temperature). Approximately 6 h after feeding most (75%) of the stomachs were less than one-quarter full and 12 h after feeding they were usually empty (75%). It seems reasonable to assume, therefore, that what was seen in the stomachs at the various collection times represented items eaten by the alevins within the previous 6 h. The 07:30 collections thus reflected feeding around dawn (05:00−06:00 E.S.T.) while the 14:30 collection represented midday feeding and the 22:00 collection reflected dusk feeding (18:00−19:00 E.S.T.).

The postemergent brook trout below the fifth falls had a mean length of 22.8 m (range 18.0−25.0 m) and a mean weight of 110 mg (range 70−150 mg); thus they were slightly shorter but heavier than the third falls population. Analyses of stomach contents revealed that *P. fuscum* larvae formed a greater proportion of the stomach contents (47%) while the importance of *B. longirostris* had fallen to 3.3%. Copepods, primarily *D. minutus*, constituted an average of 9.0% of the stomach contents, the larvae of one chironomid genus, *Micropsectra*, constituted 15.7%, but emerging chironomid adults, as a whole, contributed only 5.3%.

TABLE 2. Retention time of food in brook trout guts at 13.3°C.

Hours from capture time	No. fish examined	Fullness of stomach
0	20	$\frac{1}{4}$ to full, with 85% being over $\frac{1}{2}$ full
6	12	Empty to $\frac{1}{2}$ full, with 75% being less than $\frac{1}{4}$ full
12	12	Empty to $\frac{1}{8}$ full (pyloric section containing mostly chitinous remains), with 75% being empty. Intestine full of chitin and digested tissues
18	12	All empty. Most fish had chitin in lower end of intestine
24	12	All empty. A few fish had chitin in lower intestine
30	12	All empty. A few fish had chitin in lower intestine
48	10	All empty. Nothing in entire length of intestine or rectum

After emerging, third falls salmon alevins take up station in or close to small rapids and shallow riffle areas where they angle their large pectoral fins (Fig. 1) so that the water currents press them close to the stream bed. Thus they maintain position with a minimum output of energy (R. J. Gibson, Fisheries and Oceans, St. John's, Nfld., 1980, personal communication). At night they move into slower water near the banks and island margin.

Table 3 compares the diet of a single sample of postemergent salmon alevins with that of coexisting brook trout which were about 4 wk old; both were collected around midday on July 11, 1976. The salmon had a mean length of 25.2 mm (range 22.0−28.0 mm) and a mean weight of 176 mg (range 120−200 mg) whereas the trout were an average of 35.4 mm long (range 32.0−42.0 mm) and weighed 500 mg (range 350−890 mg). The primary food item of the salmon, in terms of volume occupied in an average stomach, appears to have been mature nymphs of the stonefly *Leuctra maria* (20.3%); however, their frequency of occurrence was only 25%, a single nymph occupying most of a stomach when present. Important both numerically and volumetrically were pupae and emerging adults of tanypodine, tanytarsine, and orthocladine chironomids and a substantial contribution (7.4% by volume) was made by larval *Hydroporus* (Coleoptera: Dytiscidae). *Leuctra maria* was also the primary component of the brook trout stomachs (26.3%) with emerging adult *Hydroptila spinata* (Trichoptera) contributing 19.9%. Emerging adult chironomids were important (12.1%) but their pupae were less important (3.8%). Large numbers of *Hydroporus* larvae were eaten but they contributed only 5.5% of the volume. No blackfly larvae and few zooplankters appeared in either diet. The former is puzzling as simuliids were abundant on the substrate and the latter was probably a consequence of normal reduced density in the river in July (Carter 1979); in some years, high rainfall at this time can cause these animals to be washed out of the lake (see Gibson and Galbraith 1975). In terms of food overlap between the salmon and trout, 23 food types were eaten in total and of these 13 (56.5%) were common to both species. However, all the major components of the diets overlapped.

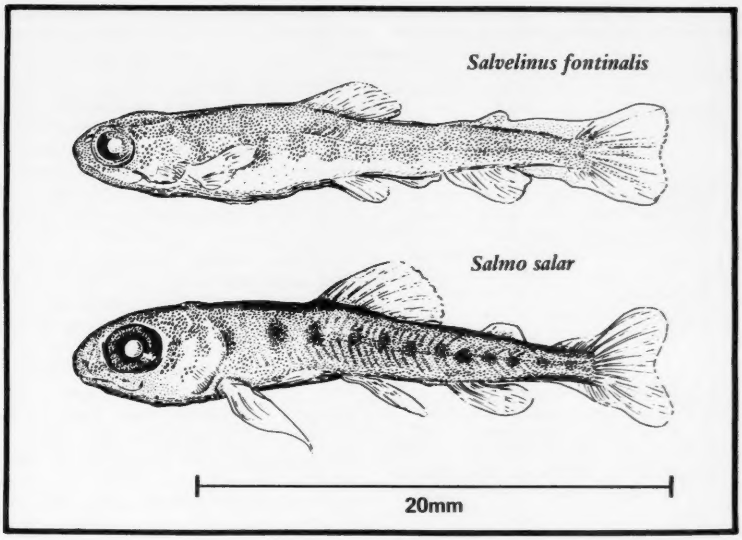

FIG. 1. Newly emerged brook trout and salmon alevins showing differences in external features.

Discussion

One of the questions which prompted this study was whether the alevins of either or both species might be feeding on stream invertebrates found interstitially (termed hyporheos) as they moved upwards to the redd surface. Dill and Northcote (1970) showed that coho salmon (*Oncorhynchus kisutch*), for example, move about both laterally and vertically within the gravel bed prior to emergence. Further, several authors have shown that some salmonid alevins emerge from the spawning gravel and reenter the interstices several times during the first few days of their free-swimming life (Neave 1955; Hoar 1976). The Matamek River is known to support a hyporheic fauna to a depth well below that at which salmonid eggs are laid (Williams and Hynes 1974). However, comparison of the first diets of the two fish species with typical hyporheic components from this river suggests that if such interstitial feeding occurs it is minimal. Evidence for this lies in the fact that most alevins collected had, unlike slightly older fishes, little material in their lower intestinal tract. It seems certain, therefore, that what was seen in the stomachs represented their first meals. Further, the food retention time data indicate that any chitin-bearing hyporheic prey eaten up to about 24 h prior to capture of the alevins would still have been retained in the lower intestine and (or) rectum; no such material was found.

For the most part brook trout in the Matamek River feed on animals passing in the water column, for example, zooplankters from the lake and drifting blackfly larvae and chironomid larvae and pupae. Drift samples showed that all but a few chironomids and the zooplankters occurred in the deeper, faster water (~1.5 m out from where the fishes were holding station). To catch these food items it would be necessary for the alevins to make short excursions to these fast-water areas. On returning to the shallows the fishes frequently have to swim upstream to their stations to counteract the diplacement by the current. This behavior was borne out by underwater observations. Adult chironomids are taken at the water surface during emergence which is when they are most susceptible to predation. The salmon similarly feed on emerging chironomids (and caddisflies) at the surface but also feed on the bottom, as evidenced by the preemergent stoneflies and beetle larve in their stomachs. They too rely heavily on a variety of chironomid larvae and pupae. They make little use of zooplankton from the lake but this is probably more a reflection of supply in July rather than choice, although zooplankton may not be as visible in fast water as in calmer areas.

Previous literature, although dealing with slightly older alevins (this is the only material available for comparison), suggests that chironomid larvae are important in the diet of young brook trout. White (1930), for example, working on Forbes Creek, Prince Edward Island, found stomachs to contain chironomids but also copepods and a few simuliid larvae. O'Connor and Power (1976) studied large fry in the Matamek watershed and concluded that chironomid larvae decreased in importance in the diet as the fishes grew. Adult dipterans other than chironomids (e.g. Simuliidae) became more important as did larval caddisflies, dragonfly, mayfly, and stonefly nymphs, and terrestrial components. Wurtsbaugh et al. (1975), on the other hand, found that recently emerged brook trout from a Californian lake ate 13% terrestrial, 11% limnetic, and 76% benthic food. Chironomid larvae and pupae were taken as part of the benthic component but mayfly nymphs were the most important item. The zooplankton (predominantly Cladocera) formed only a small part of the total diet in terms of weight but, as in the present study, represented 72% of the total numbers eaten. These same

TABLE 3. Comparison of the diets of postemergent salmon with coexisting 4-wk-old brook trout.

	S. salar (25.2 mm) (n = 20)			*S. fontinalis* (35.4 mm) (n = 20)		
Food item	Total no. found	Frequency in stomachs (%)	Avg. % vol. per stomach	Total no. found	Frequency in stomachs (%)	Avg. % vol. per stomach
Nematoda	1	5.0	0.3			
Cladocera						
Bosmina longirostris				1	5.0	0.2
Copepoda: Harpacticoida	1	5.0	0.3			
Acari	2	5.0	0.1	1	5.0	0.1
Plecoptera						
Leuctra maria	8	25.0	20.3	40	80.0	26.3
Ephemeroptera						
Ephemerella sp.	2	10.0	1.3	13	45.0	6.0
Odonata: Zygoptera						
Nymph	1	5.0	3.5	1	5.0	1.0
Trichoptera						
Hydroptila spinata	7	15.0	3.8	92	80.0	19.9
Hydropsychidae	1	5.0	0.3			
Coleoptera						
Hydroporus sp.	13	35.0	7.4	76	80.0	5.5
Terrestrial				2	10.0	2.5
Diptera: Empididae				1	5.0	0.5
Chironomidae						
Ablabesmyia sp.				16	40.0	3.7
Pentaneurini (unidentifiable)	4	15.0	7.0			
Eukiefferiella sp.	20	15.0	4.3	104	75.0	4.3
Psectrocladius sp.	29	45.0	5.0	31	55.0	2.0
Microtendipes sp.	2	10.0	0.2	1	5.0	0.1
Micropsectra sp.	2	10.0	0.4	27	40.0	1.3
Emerging adults	33	65.0	15.3	50	75.0	12.1
Pupae (unidentifiable)	35	45.0	20.3	22	20.0	3.8
Lepidoptera						
Unidentifiable larva				1	5.0	1.3
Hymenoptera						
Adult	2	10.0	1.0			
Plant fragments (terrestrial)	1	5.0	0.3	1	5.0	0.3
Unidentifiable material			8.9			9.1

authors found, in contrast, that the alevins in the lake the following year were feeding primarily on larval and pupal chironomids with few mayfly nymphs consumed. Further, the limnetic component consisted mainly of copepods, the dominant group in the zooplankton that year.

For salmon alevins, Allen (1940), working on rivers in the north of England and northern Scotland, found the most frequently selected taxa to be chironomid larvae, nymphs of *Baetis* (Ephemeroptera), and, in the case of 2nd-yr fishes only, *Ephemerella* nymphs. Nymphs of the mayfly *Ecdyonurus* and the stonefly *Leuctra* were seldom eaten although they dominated the benthos. Lillehammer (1973a), in contrast, found that the dominant benthic forms, chironomids and stoneflies in the River Suldalslagen, Norway, were also the most important food items eaten by fishes of between 2.5 and 3.0 cm in June. Planktonic crustaceans, most numerous in the river in August, were the most common food items in August and September.

Lillehammer (1973b) stated that the same species of fish may take different food in different parts of a water body. He gave an example of one river where the salmon were found feeding from the drift and the trout (*S. trutta*) from the bottom while in a nearby stream the reverse took place. This strongly suggests that food availability is the primary factor controlling what is or is not ingested. Mundie (1971), studying the consumption of chironomids by coho fry, pointed out that fry are not uniformly distributed in streams but frequently collect in back eddies. In such places, 85% of the total drift occurring in the stream may be out of their reach. His data support the idea that availability and not abundance is the important factor. Calculation of forage ratios showed greater consumption of chironomids (pupae, adults, larvae, and exuviae) than would have been expected from their proportion in the drift (see also Frost and Went 1940). However, should an available food item become more abundant then it will surely be eaten more frequently; this is borne out by the increased consumption of *P. fuscum* and *Micropsectra* larvae by brook trout at the fifth falls where their benthic densities are greater than at the third falls (Gibson and Galbraith 1975). Availability may be manifest in several forms including food shape, size, color, and mobility (Kislalioglu and Gibson 1976; Rimmer and Power 1978).

In the Matamek River, competition for food between the two salmonid species appears reduced through the trout emerging ~1 mo before the salmon. Dickson (1980) concluded that this staggered emergence favors the trout because the growth advantage from the 0+ year-class is enough to maintain the growth differential in favor of the trout for the rest of the time the two species cohabit the river. Although the trout have no obvious competitors for food when they first emerge (based on underwater surveys at the study sites), the salmon do (Table 3). However, Gibson (1973) has shown that, at least when juveniles, salmon are more aggressive than brook trout. Possibly, therefore, even when newly emerged they may be able to hold their own against the older trout and, of course, they do occupy a slightly different microhabitat. At this stage in their lives, although the same length as newly emerged trout, the salmon have larger mouths (salmon mean gape 3.20 mm, range 2.9−3.5 mm, n = 20; trout mean gape 2.61 mm, range 2.1−3.1 mm, n = 15) (Fig. 1). This feature enables them to feed on much larger food items than do trout of comparable age and may partially account for their eating the same food (e.g. large stonefly nymphs) as 4-wk-old trout (mean gape 5.05 mm, range 4.5−6.0 mm, n = 20).

In conclusion, it is evident that in the Matamek River the first diets of brook trout are primarily derived from small- to medium-sized aquatic invertebrates in the water column and on the surface in both slow- and fast-water areas, although the alevins hold station in the former. In river systems which do not have a headwater lake, the lack of zooplankton would probably be compensated for by increased consumption of drifting benthic species. The salmon alevins hold station in faster water and tend to feed more from the substrate on medium- to large-sized invertebrates, although items at the water surface may be taken also. Lack of zooplankton in their diet probably reflects availability at that time.

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Avoidance Reactions of Juvenile Coho Salmon (*Oncorhynchus kisutch*) to Monocyclic Aromatics

DESMOND J. MAYNARD[1,2] AND DOUGLAS D. WEBER

Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

MAYNARD, D. J., AND D. D. WEBER. 1981. Avoidance reactions of juvenile coho salmon (*Oncorhynchus kisutch*) to monocyclic aromatics. Can. J. Fish. Aquat. Sci. 38: 772–778.

Presmolt coho salmon (*Oncorhynchus kisutch*) tested in a Y-maze avoided a mixture of monocyclic aromatic hydrocarbons at concentrations of 3–4 mg/L while smolting coho salmon avoided concentrations of less than 2 mg/L. Three components (benzene, toluene, *o*-xylene) of the mixture were tested individually with presmolt salmon and each was avoided at a lower concentration than when compared with the total hydrocarbon concentration of the mixture; *o*-xylene was most repellent, causing statistically significant avoidance at a concentration of 0.2 mg/L. Electrophysiological recordings from the olfactory bulb indicated that short-term exposure of juvenile coho salmon to monocyclic aromatic hydrocarbons at 4 mg/L did not disrupt this chemosensory modality; the olfactory system was stimulated by an aromatic hydrocarbon mixture at concentrations correlating with observed avoidance behavior.

Key words: salmon, behavior, avoidance, monocyclic aromatic hydrocarbons, *Oncorhynchus*, olfactory EEG

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Placé dans un labyrinthe en Y, au stade de présmolt, le saumon coho (*Oncorhynchus kisutch*) a évité un mélange de 3 à 4 mg/L d'hydrocarbures aromatiques monocycliques; au stade de smolt, il a su éviter des concentrations inférieures à 2 mg/L. Trois composés (benzène, toluène, *o*-xylène) du mélange ont été essayés séparément sur le présmolt qui, dans chaque cas, les a évités à une concentration inférieure à la concentration totale des hydrocarbures du mélange. L'*o*-xylène a été le plus répulsif avec un évitement statistiquement significatif à 0,2 mg/L. D'après les enregistrements électrophysiologiques du bulbe olfactif, cet organe du coho juvénile exposé brièvement à des hydrocarbures aromatiques monocycliques (4 mg/L) n'a pas perdu sa sensibilité. Le système olfactif a été stimulé par un mélange d'hydrocarbures aromatiques à des concentrations qui correspondaient à la réaction d'évitement observée.

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IT has been generally assumed that salmon will avoid petroleum-contaminated waters; however, several studies indicate that this is not necessarily true. During the course of Morrow's (1973) experiments with crude oil, juvenile coho salmon (*Oncorhynchus kisutch*) did not avoid oil but actually swam in the oil film. Similar observations for juvenile chinook salmon (*O. tshawytscha*) were reported by Bean et al. (1974). Preliminary studies in our laboratory also indicate that juvenile coho do not avoid fresh Prudhoe Bay crude oil either on the water surface or dispersed in the water column (Maynard 1980).

Studies concerned with avoidance of specific hydrocarbons by salmonids have shown varied results. Folmar (1976) found that rainbow trout (*Salmo gairdneri*) tested in a Y-maze were attracted to 0.01 mg of *p*-xylene per litre but avoided 0.1 mg/L. In a study by Sprague and Drury (1969), rainbow trout did not avoid phenol at a near lethal concentration of 10 mg/L. Rice (1973) found that avoidance of the water-

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[1] Author to whom all correspondence should be addressed.

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soluble fraction of Prudhoe Bay crude oil by pink salmon (*O. gorbuscha*) fry varied with stage of fish development, temperature, and salinity.

Though monocyclic aromatic hydrocarbons make up less than 10% of most crude oils, their high water solubility, their extensive use in the manufacture of petrochemical products, and their use as carrier solvents for a variety of pesticides and as aquatic herbicides cause them to be common environmental contaminants. In addition, these aromatic hydrocarbons are considered to be some of the most toxic of petroleum components. Toluene, benzene, and xylenes cause narcosis, central nervous system disruption, and profound circulatory changes in vertebrates (Thienes and Hailey 1972), and benzene has been reported to be a carcinogen (Snyder and Koscis 1975).

This laboratory study concerns the avoidance response of juvenile coho salmon to petroleum hydrocarbons, especially the monocyclic aromatic compounds. The purpose was to determine the relationship of avoidance response to different concentrations of the compounds and how the response is affected by age, season, and behavioral state. The results of a similar study with migrating adult salmon under field conditions are reported elsewhere in this issue (Weber et al. 1981).

Methods

Juvenile coho salmon were 6–10 cm in fork length and from two sources within the Puget Sound region of Washington State. A 1977 brood (year of hatching) stock of coho of Issaquah Creek origin (eastern Puget Sound) was used for all avoidance tests conducted through July 1978. A mixed 1978 brood year stock consisting of Issaquah Creek and Minter Creek (southern Puget Sound) coho were used in avoidance tests conducted after August 1978. All fish were maintained under standard hatchery conditions and tested in freshwater at ambient temperature which varied seasonally from 5 to 17°C.

The monocyclic aromatic hydrocarbons introduced into the water for avoidance testing consisted of a mixture (Table 1) modeled on the saltwater-soluble fraction (SWSF) of fresh Prudhoe Bay (PBCO) and contained 95% (by weight) of those hydrocarbons detected in the SWSF. The hydrocarbon components were determined from five samples of the SWSF of PBCO generated by the method of Roubal et al. (1977) and the concentration of each component was found to be similar to that reported in detail by Roubal et al. (1978). Samples (100 mL) of the SWSF were extracted on the day of collection using 5 mL of carbon disulfide. The organic phase was separated, evaporated to 0.8 mL, and 8 μL of the extract was gas chromatographed on a 1-m- long by 2-mm-ID glass column packed with 0.2% Carbowax 1500 on 60/80 mesh Carbopack C (Supelco, Bellefonte, PA).[3] Nitrogen was used as a carrier gas with a flow rate of 46 mL/min and temperature programming was from 40 to 215°C at 15°/min. All data were corrected for extraction efficiency and no petroleum hydrocarbons were detected in control water samples. The identities of hydrocarbon components were confirmed by mass spectral analysis on a single sample using methods described by MacLeod et al. (1977).

TABLE 1. Composition of mixture of monocyclic aromatic hydrocarbons used in testing juvenile coho salmon.

Hydrocarbon	% by weight in mixture[a]
Toluene	57.0
o-Xylene	16.2
Benzene	7.9
1,2,4-Trimethylbenzene	7.3
p-Xylene	4.8
m-Xylene	4.8
Ethylbenzene	2.0

[a]Hydrocarbons used were of analytical or spectrophotometric grade.

In generating a water–hydrocarbon mixture, water was passed through a water jet eductor (Schutte and Koerting, Cornwells Heights, PA, type 264, 1.3 cm, capacity ratio of 0.36)[3] at a flow rate of 16 L/min and a pressure of 5.5 kg/cm^2 (Fig. 1). The hydrocarbon mixture and individual components were introduced into the vacuum port of the eductor by a calibrated, continuous-flow, syringe pump; the reduced pressure in the eductor port vaporized the hydrocarbon mixture prior to its mixing with water. A 50 × 7.5 cm glass column filled with 3-mm glass beads was inserted in the line after the eductor to even out minor fluctuations in hydrocarbon injection.

An opaque all-glass Y-maze was used for avoidance testing (Fig. 1). Equal water flow (16 L/min) was maintained in both test and control arms with water lines arranged so that the test and control arms could be alternated after each test. At initiation of each avoidance test, 20–60 previously untested fish were placed in the holding area of the Y-maze for 15 min before hydrocarbons were introduced into one arm. The perforated Teflon gate was then raised, allowing fish equal access to either arm, and after 60 ± 10 min the gate was lowered and the number of fish in each arm recorded. Control tests were conducted without hydrocarbons present in either arm of the Y-maze.

We assumed that if fish did not avoid water with aromatic hydrocarbons present, they would leave the holding area and enter each arm of the Y with an expected frequency determined by control tests (tested by chi-square). Linear regressions of the avoidance data were calculated and *r* values tested for levels of significance. Linear estimates were utilized to obtain 50% avoidance levels (AL50) representing the hydrocarbon concentrations at which half the expected number of fish would be found in the test arm.

The effectiveness of the eductor in solubilizing the mixture of monocyclic hydrocarbons in the water was determined by gas chromatographic (GC) analysis of replicate water samples taken at 20-min intervals from the test arm of the Y-maze with known injection rates of the model mixture set on the calibrated syringe pump. Because the solubilizing system introduced hydrocarbons into water precisely (Fig. 2), concentrations of hydrocarbons present during avoidance testing were based on calculted values.

Standard electrophysiological techniques were used in recording electrical activity from the olfactory bulb of juve-

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[3]Mention of commercial productions is for information only and does not constitute endorsement by the U.S. Department of Commerce.

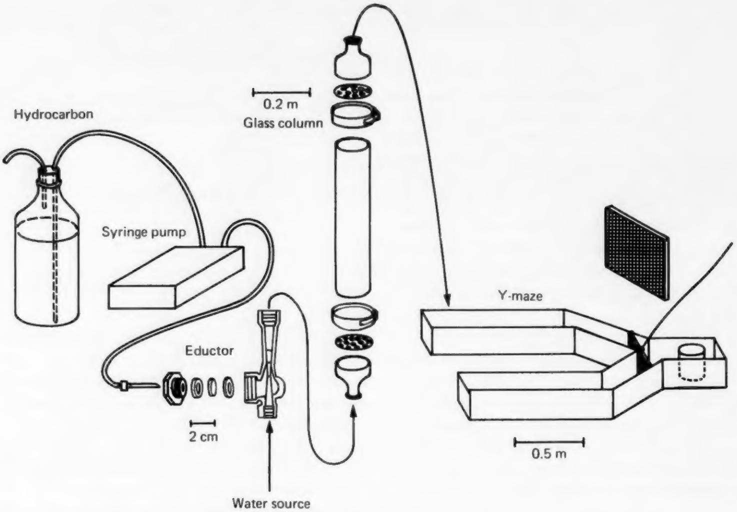

FIG. 1. System used for introduction of hydrocarbons into water and apparatus used in testing avoidance of petroleum hydrocarbons by juvenile coho salmon.

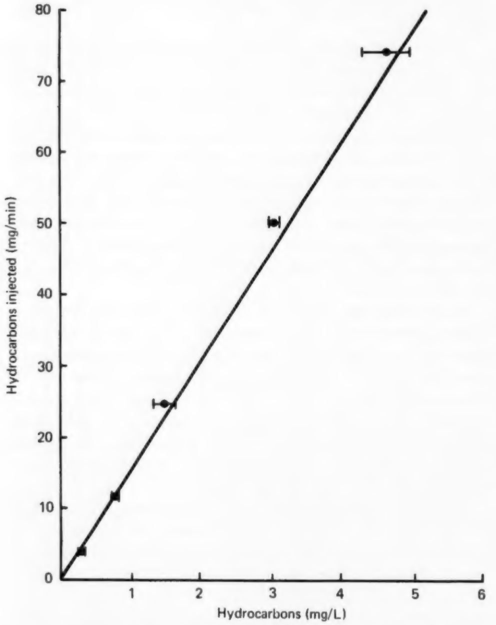

FIG. 2. Amount of aromatic hydrocarbon mixture injected into the solubilizing system and concentration (milligrams per litre) of mixture present in the water as calculated from water flow (line) and by GC analysis (circles, average GC value). Horizontal bars represent standard error of GC values.

nile coho salmon (1976 brood year, Skagit River, Washington stock, 20 cm average length; for method see Hara 1973; Bodznick 1975; Maynard 1980). Control water and water used to make L-serine solutions were obtained from the uncontaminated arm of the Y-maze; hydrocarbon solutions were obtained from the test arm. Hydrocarbon solutions were placed in Teflon wash bottles and replenished at least every 30 min. To assess detection of aromatic hydrocarbons, we obtained electroencephalographically (EEG) the response of the olfactory bulb to different hydrocarbon concentrations introduced in the nares for 6−10 s. Olfactory disruption as a result of aromatic hydrocarbon exposure was evaluated by recording the EEG response to a solution containing 10^{-3} mol L-serine per litre both before and after rinsing the nares continuously for 6−10 s, 10 min, or 20 min with a solution containing 4.0 mg of the aromatic hydrocarbon mixture per litre. The EEG responses were quantified by measuring the area under the integrated EEG signal for the first 2−6 s following stimulation of the nares. Relative changes in amplitude of the EEG response were analyzed using an unpaired *t*-test for detection experiments and matched-pair *t*-test for disruption experiments.

Results

In all control tests coho consistently preferred the right arm of the Y-maze (average 59%) and there was no significant difference ($P > 0.05$) between control tests using salmon of 1977 or 1978 brood years. Coho tested in the summer avoided the aromatic hydrocarbon mixture at less than half the concentration avoided in the winter (Table 2). Also, individual aromatic hydrocarbons were generally avoided at lower concentrations than the total concentration of the mixture.

TABLE 2. Hydrocarbon concentration (treated arm) and number of coho salmon present in each arm of Y-maze (fish remaining in holding area not included) (***$P < 0.001$; **$P < 0.05$).

Fish group Season Month(s)	Water temp. (°C) (range)	Hydrocarbons		No. of tests	Number (*n*) and percentage of fish in each arm of Y-maze								Significance probability
					Left (treated)		Right (control)		Left (control)		Right (treated)		
		Species	Concn. (mg/L)		*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	
Control[a]	11.0 (±6.3)		0	14			155	(61)	101	(39)			
Test Winter Jan.–Feb.	6.9 (±1.3)	Mixture	0.9	12	32	(45)	39	(55)	57	(45)	69	(55)	ns[b]
			1.9	8	35	(50)	35	(50)	31	(47)	35	(53)	ns
			2.8	7	23	(38)	38	(62)	20	(36)	35	(64)	ns
			3.7	8	19	(25)	58	(75)	44	(83)	9	(17)	***
			4.6	8	5	(08)	60	(92)	60	(80)	15	(20)	***
Test Summer June–July	16.4 (±2.0)	Mixture	1.4	8	38	(34)	73	(66)	55	(60)	37	(40)	***
			1.9	8	51	(35)	93	(65)	73	(74)	26	(26)	***
			2.8	8	5	(07)	69	(93)	114	(83)	23	(17)	***
Test Winter Feb.–Mar.	7.8 (±0.4)	Toluene	1.4	9	44	(40)	65	(60)	50	(60)	33	(40)	**
			1.8	6	9	(21)	35	(79)	68	(89)	8	(11)	***
Control[c]	11.5 (±4.3)		0	11			124	(57)	92	(43)			
Test Autumn Nov.	12.0 (±1.5)	Toluene	0.9	6	29	(56)	23	(44)	31	(53)	27	(47)	ns
			1.8	6	11	(35)	20	(65)	51	(71)	21	(29)	***
			2.8	4	5	(13)	35	(87)	34	(94)	2	(06)	***
Test Autumn Nov.–Dec.	8.3 (±1.6)	*o*-Xylene	0.2	6	14	(23)	48	(77)	27	(60)	18	(40)	***
			0.5	6	14	(24)	44	(76)	24	(59)	17	(41)	**
			1.0	4	3	(17)	15	(83)	40	(95)	2	(05)	***
			1.9	4	3	(06)	44	(94)	36	(84)	7	(16)	***
			2.9	4	1	(02)	51	(98)	30	(83)	6	(17)	***
Test Autumn Nov.–Dec.	8.3 (±1.6)	Benzene	0.9	4	16	(42)	22	(58)	17	(43)	23	(57)	ns
			1.9	4	9	(27)	24	(73)	27	(69)	12	(31)	**
			2.8	4	0	(0)	39	(100)	39	(97)	1	(03)	***

[a] Total tests, various times of year; western Puget Sound stock (1977 brood year). Control tests were interspersed between hydrocarbon tests. Standard error for control tests was ±5.0%.

[b] ns, not significant.

[c] Total tests, various times of year; western–eastern Puget Sound stock mixture (1978 brood year). Control tests were interspersed between hydrocarbon tests. Standard error for control tests was ±5.9%.

Comparison of avoidance at different times of year with individual and a mixture of aromatic hydrocarbons is based on AL50 values; these values were calculated from regression equations based on results of individual avoidance tests (Table 3). Of the aromatic hydrocarbons tested in November and December of 1978, the AL50 for *o*-xylene is approximately half (0.7 mg/L) that observed for either toluene or benzene (1.8 mg/L). Avoidance of the mixture of aromatic hydrocarbons is dissimilar to avoidance of individual hydrocarbon species comprising the mixture; thus, there appears to be an interactive effect when the hydrocarbons are mixed together, suggesting that the combination of hydrocarbons

TABLE 3. Linear regression equations calculated from concentration of hydrocarbons in each arm of Y-maze and percentage fish present in respective arms. AL50 represents the hydrocarbon concentration avoided by 50% of the fish (**$P < 0.05$).

Hydrocarbon	Treated arm	No. tests	Regression equation ($Y =$)[a]	Significance probability	AL50 (mg/L)	AL50 (avg.)
Mixture (Jan.−Feb.)	Left	12	$81.44 - 15.08X$	**	3.66	3.71
	Right	11	$85.66 - 13.88X$	**	3.75	
Mixture (June−July)	Left	11	$51.86 - 12.99X$	ns[b]	1.98	1.89
	Right	13	$56.76 - 12.88X$	**	1.80	
Toluene (Nov.)	Left	7	$63.04 - 15.15X$	**	2.34	1.99
	Right	9	$61.09 - 17.48X$	**	1.65	
o-Xylene (Nov.−Dec.)	Left	12	$33.30 - 9.43X$	**	0.60	0.68
	Right	12	$40.07 - 10.27X$	**	0.76	
Benzene (Nov.−Dec.)	Left	6	$69.68 - 24.18X$	**	1.74	1.75
	Right	6	$73.29 - 23.38X$	**	1.75	

[a] Percentage fish arcsin transformed.

[b] ns, not significant.

TABLE 4. Comparison of temperature, developmental stage, and percentage juvenile coho salmon present in right arm of Y-maze when aromatic hydrocarbon(s) is present in same arm.

Months	Hydrocarbon	Concn. (mg/L)	Temp. (°C)	Developmental stage	% in right arm of Y-maze
Jan.−Feb.	Mixture	2.8	6.9	Presmolt	64.0
June−July	Mixture	2.8	16.4	Late smolt	17.0
November	Toluene	1.8	12.0	Presmolt	29.17
Feb.−Mar.	Toluene	1.8	7.8	Early smolt	10.53

TABLE 5. Stimulatory effectiveness of a mixture of monocyclic aromatic hydrocarbons on the olfactory bulb EEG as measured by amplitude of response compared with control water and as percentage of standard 10^{-3} mol L-serine per litre response (***$P < 0.001$).

Hydrocarbon mixture (mg/L)	No. of tests[a]	Avg. amplitude of EEG response (SD)[b]		Significance probability	% of 10^{-3} mol/L L-serine EEG response (avg.)
		Control	Test		
1.9	10	37 (27)	23 (24)	ns[c]	7
2.8	20	35 (24)	87 (52)	***	25
3.7	15	31 (19)	117 (49)	***	32

[a] Equal numbers in each of control and test groups.

[b] Relative values calculated from area of integrated EEG response for 2−6 s after introduction of stimulant to nares (in centimetres squared).

[c] ns, not significant.

results in an odor or taste distinct from that of the individual components (Amoore 1970).

Presmolt coho salmon did not significantly avoid a mixture containing 2.8 mg of aromatic hydrocarbons per litre in January−February but 4 mo later smolts significantly avoided this same hydrocarbon concentration (Table 4). Similar responses were found using toluene, with a greater percentage of the fish avoiding 1.8 mg toluene per litre as early smolts than as presmolts. Consistently more smolts avoided a given concentration of aromatic hydrocarbons than did presmolts regardless of water temperature.

Electrophysiological recordings from the olfactory bulb of juvenile coho salmon were conducted in the winter months and used as a criterion of detection of aromatic hydrocarbons by the olfactory system. Amplitudes of electrical activity of the olfactory bulb (EEG respones) following exposure of the nares to control water and a standard stimulant (10^{-3} mol L-serine per litre) were compared with EEG responses induced by different concentrations of the aromatic hydrocarbon mixture (Table 5). An increase in EEG response to the aromatic hydrocarbon mixture occurred at concentrations between 1.9 and 2.8 mg/L; this is less than the 2.8−3.7 mg/L

TABLE 6. Difference in olfactory bulb EEG response to 10^{-3} mol L-serine per litre before and after rinsing nares with a 4.0-mg/L mixture of monocyclic aromatic hydrocarbons.

	Duration of hydrocarbon mixture rinse (min)		
	<1	10	20
Difference from previous serine response (mean ± SE)[a]	6.4±16.7	42.7±17.5	−36.2±34.1
No. of fish tested	6	6	5
Significance probability	ns[b]	ns	ns

[a] Relative values calculated from area of integrated EEG response for 2−6 s after introduction of L-serine (in centimetres squared).

[b] ns, not significant.

concentrations which resulted in significant behavioral avoidance of the hydrocarbons by coho salmon tested at the same time of year and ambient temperatures.

Coho salmon whose nares were exposed to a mixture containing 4.0 mg of aromatic hydrocarbons per litre for up to 20 min showed no significant difference in EEG response to 10^{-3} mol L-serine per litre given before and after exposure (Table 6).

Discussion

Differences in avoidance of monocyclic aromatic hydrocarbons by juvenile coho salmon with season and stage of development is expected, as similar behavioral variations have been reported for other salmonids exposed to a variety of stimuli. Northcote (1958) observed that rainbow trout held at 16-h day lengths exhibited negative rheotaxis whereas trout held at 8-h day lengths were positively rheotactic. Northcote attributed these rheotactic changes to seasonal movement of trout from outlet to inlet streams of rearing lakes. McInerney (1964) examined salinity preference of five species of juvenile Pacific salmon and found that initially the salmon preferred freshwater but they gradually preferred more saline waters at smoltification. The timing of increased salinity preference related extremely well with timing of seaward migration. Rice (1973) observed that pink salmon fry avoided the water-soluble fraction of PBCO in salt water at a concentration of approximately one magnitude less than they did in freshwater.

The coho salmon used in this study were maintained and tested under ambient temperatures and photoperiods, conditions similar to the environment in which the fish would be found naturally. As presmolts, coho display both territoriality and dominance hierarchies (Shaplov and Taft 1954; Chapman 1962; Mason 1966; Allee 1974). Near the end of their 1st yr, in late February, local juvenile coho reared under natural conditions approach smoltification and start seaward migration. Peak downstream migration is from mid-April through May and terminates in July. A plausible explanation for differential avoidance of petroleum hydrocarbons by presmolt and smolting coho salmon is that presmolts, due to their aggressiveness and territoriality, resist displacement, thus tolerating a higher concentration of pollutant compared with smolting coho which under natural conditions are undergoing displacement during their seaward migration.

There are several reports concerning the acute toxicity of crude oil to juvenile Pacific salmon (Bean et al. 1974; Morrow 1973; Korn et al. 1979; Rice 1973; Rice et al. 1975; Rice et al. 1976; Moles et al. 1979). Of these reports the most pertinent to our study with respect to oil type, method of chemical analysis, and species tested is that conducted by Moles et al. (1979). Juvenile coho and chinook salmon were exposed to the water-soluble fraction of PBCO and the 96-h median tolerance limit (TLm) values were based on total aromatic hydrocarbons as determined by GC analysis. The 96-h TLm was 3.6 mg/L at 8°C for coho and 3.6 mg/L at 6°C for chinook salmon. Our results suggest that over 50% (AL50 of 3.71 mg/L) of the presmolt coho salmon would not avoid a potentially toxic concentration of petroleum hydrocarbons.

Because the aromatic hydrocarbon mixture used in our avoidance studies consisted only of monocyclic aromatics, a more appropriate comparison would be to relate avoidance to toxicity of individual monocyclic aromatic hydrocarbons. Brockson and Bailey (1973), using juvenile chinook in a static system, report that acute toxicity for benzene was 15 mg/L (duration of exposure not stated by authors but presumably was 96 h). Morrow et al. (1975), in studies with juvenile coho salmon, showed that 10 mg/L concentrations of toluene, xylene, benzene, or ethylbenzene produced no significant mortalities in 96 h but that 50 mg/L concentrations of toluene, benzene, and ethylbenzene, or a 100 mg/L concentration of xylene produced 100% mortality in 96 h. Moles et al. (1979) exposed juvenile Pacific salmon to benzene and observed a 96-h TLm of 11.7 mg/L for chinook salmon and 14.1 mg/L for coho salmon. Korn et al. (1979) found a lower 96-h TLm for pink salmon fry exposed to toluene: 6.4 mg/L at 4°C and 8.1 mg/L at 12°C. Walsh et al. (1977), using a flow-through system, found that rainbow trout exposed to xylene for 2 h survived a concentration of 7.1 mg/L but suffered a 100% mortality at 16.1 mg/L, with an estimated 96-h LC50 of 13.5 mg/L. In all these studies where 24- and 96-h TLm values were presented the TLm hydrocarbon concentrations are similar. In general, it appears that juvenile salmonids would suffer acute mortalities when exposed to 5−15 mg/L concentrations of either benzene, toluene, xylene, or ethylbenzenes. In our study less than 20% of the fish were found in the treatment arm when the concentration of the individual monocyclic aromatics was 3 mg/L.

Comparison of EEG disruption data with results of similar studies using other pollutants indicates that the aromatic hydrocarbon mixture did not detrimentally affect olfaction. When Hara (1972) infused the nares of juvenile coho and sockeye salmon for 10 s with either 2.7 mg $HgCl_2$ per litre or 1.6 mg $CuSO_4$ per litre the olfactory response to all stimulants was eliminated. Also, Hara et al. (1976) found a significant decrese in rainbow trout EEG response to stimulants after 30 min of exposure to 0.3 mg $HgCl_2$ per litre.

The positive results of the EEG experiments do not rule out the use of other sensory modalities to detect and avoid aromatic hydrocarbons. Also, the olfactory bulb EEG response is the result of integrated neural activity; thus, these results do not necessarily reflect the minimal olfactory detection of aromatic hydrocarbons.

In general, our results suggest that juvenile salmon would avoid acute lethal concentrations of monocyclic aromatic hydrocarbons presented individually; however, there is some question as to whether they would avoid toxic concentrations of these compounds found in the water-soluble fraction of crude oil. It should be noted that there is no published information on behavior and toxicity following chronic low level exposure or on the consequences of avoidance, particularly if avoidance results in change of habitat.

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Avoidance Reactions of Migrating Adult Salmon to Petroleum Hydrocarbons

DOUGLAS D. WEBER,[1] DESMOND J. MAYNARD,[2] WILLIAM D. GRONLUND, AND VYACHESLAV KONCHIN[3]

Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

WEBER, D. D., D. J. MAYNARD, W. D. GRONLUND, AND V. KONCHIN. 1981. Avoidance reactions of migrating adult salmon to petroleum hydrocarbons. Can. J. Fish. Aquat. Sci. 38: 779–781.

Mature Pacific salmon (*Oncorhynchus* sp.) migrating upstream during the peak of the run substantially avoided a mixture of monocyclic aromatic hydrocarbons in the water at concentrations of 3.2 mg/L and higher.

Key words: salmon, behavior, avoidance, monocyclic aromatic hydrocarbons, *Oncorhynchus*

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Au plus fort de sa remontée, le saumon mature du Pacifique (*Oncorhynchus* sp.) a, dans une forte proportion, évité les eaux qui contenaient 3,2 mg/L ou plus d'un mélange d'hydrocarbures aromatiques monocycliques.

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AVOIDANCE of an oil-contaminated area by highly mobile fish species may significantly reduce the impact of the pollutant. Conversely, for species such as Pacific salmon (*Oncorhynchus* sp.), avoidance of certain areas (e.g. migratory routes and home spawning streams) where oil is present could be severely detrimental.

Experiments with juvenile salmon indicate that downstream migrants would probably avoid acutely lethal concentrations of petroleum hydrocarbons (Maynard and Weber 1981; Rice 1973). There is, however, some question concerning the applicability of their data to adult salmon, particularly at the time of the salmon's return to the "home" stream to spawn. Pacific salmon, during their spawning migration, have an appetitive "homing" behavior which may override any aversion to petroleum contaminants shown by younger fish.

The objective of this study was to determine the effect of petroleum hydrocarbons on adult salmon migration and to relate the results to laboratory studies on avoidance of petroleum hydrocarbons by juvenile salmon.

Methods

The study site was located on Chambers Creek which enters southern Puget Sound, Washington. A tidewater dam having a central spillway, ladders on each side, and trapping facilities at the upstream head of each ladder is located 1 km from the mouth of the creek. The average discharge of the creek during November was 1.8 m^3/s with ~12% of the water passing through the west fish ladder, 28% passing through the east ladder, and the remainder passing over the spillway. Water temperature was 9–10°C. In testing avoidance behavior of adult migrating salmon, a mixture of aromatic hydrocarbons was introduced at the head of the west ladder; the east ladder was left in its normal condition.

The mixture of monocyclic aromatic hydrocarbons introduced into the water contained the following percentages of individual components by volume: benzene, 7.9%; toluene, 57.0%; ethylbenzene 7.0%; *m*- and *p*-xylene, 23.3%; and *o*-xylene, 4.8%. This mixture closely approximated, but differed slightly from, the water-soluble fraction of Prudhoe Bay crude oil in the relative proportions of monocyclic aromatic hydrocarbons (Maynard and Weber 1981).

In generating the water–hydrocarbon mixture, water from the dam impoundment was pumped through a water jet eductor (Schutte and Koerting, Corvallis Heights, PA, type 264, 1.3 cm, capacity ratio of 0.36)[4] at a flow rate of 12 L/min and a pressure of 6.7 kg/cm^2. The hydrocarbon mixture was introduced into the vacuum port of the eductor at flow rates of 3–105 mL/min. The eductor vacuum served as a pump and hydrocarbon flow was regulated by a calibrated metering valve. From the eductor the water–hydrocarbon mixture was piped to the top of the west fish ladder where a diffuser pipe, with its outlet 0.6 m below the water surface, provided dispersion of the water–hydrocarbon mixture across the width

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[1] Author to whom all correspondence should be addressed.

[2] Based in part on a thesis submitted in partial fulfillment of requirements for the Masters degree at the College of Fisheries, University of Washington, Seattle.

[3] Visiting scientist, Department of Biology, Moscow State University, Moscow, USSR.

[4] Mention of commercial products is for information only and does not constitute endorsement by the U.S. Department of Commerce.

of the ladder with further mixing taking place in the water turbulence in the ladder.

Prior to the initiation of an avoidance test, the salmon in the traps at the head of each ladder were counted and placed upstream; water depth in the top step of the west ladder was measured and the flow calculated using the Francis, sharp-crested weir formula (King 1954). The duration of each test varied from 4.5 to 22 h depending upon the numbers of migrating fish, stream discharge, and tidal fluctuation.

At the termination of each test, the amount of hydrocarbon mixture used was measured; duplicate water samples for chemical analysis were taken from the top of the west ladder into which the hydrocarbons were introduced and from the middle of the ladder as tidal height permitted (a water sample was also collected from the top of the east ladder and served as control); and the numbers of fish in each trap were counted by species and placed upstream.

Water samples were collected in 315-mL glass bottles containing 3 mL of concentrated hydrochloric acid and the hydrocarbons were extracted within 3 d after collection. The water sample was spiked with a recovery standard and extracted three times with a total of 24 mL of CH_2Cl_2. The extract was dried with Na_2SO_4 in a concentrator tube and reduced to 0.8 mL. A gas chromatographic (GC) internal standard was added and the extract analyzed by glass capillary−gas chromatography (characteristics described by MacLeod et al. 1977). The limit of detection for individual aromatic compounds was 1 μg/L. Because benzene eluted with the solvent front, the calculated concentration of total hydrocarbons present in the sample was increased by 8% (i.e. equals percentage benzene present in the mixture and assumes similar solubility and volatility as the other monocyclic aromatic hydrocarbons in the mixture). All concentrations were corrected for extraction efficiency. No petroleum hydrocarbons were detected in control samples.

Results

Control observations (i.e. salmon migrating up the east or west fish ladders when aromatic hydrocarbons were not added) and tests with hydrocarbons were made during the peak of salmon migration in Chambers Creek. More than 1400 salmon ascended the dam during the control periods and of these 56% chose the west ladder (Table 1). Another 1149 salmon migrated over the dam during the test periods when hydrocarbons were added to the water in the west fish ladder.

Of the total number of salmon ascending the two fish ladders during the study period, 99% were coho salmon (*Oncorhynchus kisutch*) and the remaining 1% were a mixture of pink (*O. gorbuscha*), chum (*O. keta*), and chinook (*O. tshawytscha*).

Data were analyzed using hydrocarbon concentrations based on GC analyses rather than the ~40% greater concentrations calculated from the amount of hydrocarbon mixture used and water flow. The Francis formula used for calculating water flow in the fish ladder is a rough approximation of weir discharge and tends to underestimate water volume at high approach velocities representative of the field study site. Considering the consistency of hydrocarbon concentrations in replicate water samples taken at the top and in the middle of the fish ladder we elected to use the results of chemical analysis as being the more appropriate for representing the hydrocarbon concentration in the water that the migrating salmon encountered. The hydrocarbon concentration at which 50% of the fish which were expected to ascend the west ladder avoided it (AL50) was 3.2 mg/L; the AL50 value was calculated

TABLE 1. Numbers of salmon ascending fish ladders of Chambers Creek dam when aromatic hydrocarbons were, and were not (control), present in water of west ladder.

Date	Observation and test period(s) (h)	Salmon ascending ladders			Hydrocarbon concn. in ladder (μg/L)		
			%			GC analysis (duplicate samples)	
		Total	East	West	Calculated[a]	Top of ladder	Middle of ladder
Control							
Oct. 29− Nov. 15	6−24	1431	44	56	0		
			(SD = 7.8, n = 9)				
Test							
Nov. 2	22.0	236	56	45	1900	1018, 1580	[b]
Nov. 3	6.0	107	69	31	4900	2414, 2968	2553, 3273
Nov. 8	18.5	56	75	25	5300	2095, 2161	1961, 2289
Nov. 11	7.0	59	64	36	2700	1355, 1625	950[c]
Nov. 12	16.0	139	55	45	1900	987, 1323	971[c]
Nov. 13	4.5	35	71	29	4400	2487, 2509	2218, 2284
Nov. 13	6.0	61	93	7	6100	3638, 3858	3715[c]
Nov. 14	5.0	126	56	44	1000	476, 582	[b]
Nov. 16	5.0	100	69	31	3800	1960, 2188	2059, 2105
Nov. 17	19.5	149	45	55	300	175, 177	175[c]
Nov. 18	20.0	60	57	43	2600	1458, 1592	1351, 1477
Nov. 19	6.0	21	95	5	7000	4436, 4734	3925, 3985

[a] Based on millilitres of hydrocarbon mixture used per minute and water flow in fish ladder.

[b] No water sample collected.

[c] No replicate water sample collected.

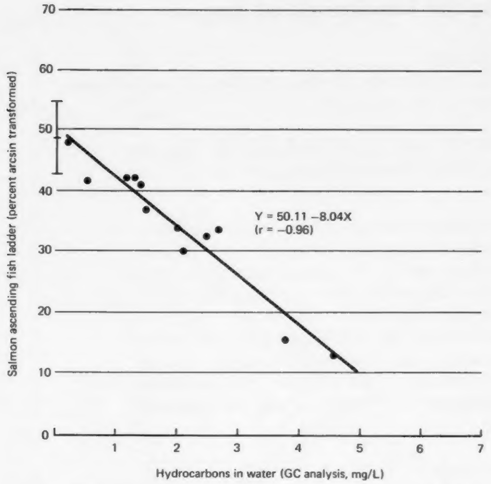

FIG. 1. Percentage of adult salmon migrating up (west) fish ladder and concentration of aromatic hydrocarbons in the water as determined by GC analysis. Points represent averages using duplicate water samples taken from top of ladder. Mean and standard deviation for control observations are given at left. Percentage fish ascending ladder was arcsin transformed.

from a regression equation based on results of individual avoidance tests (Fig. 1). Water samples for hydrocarbon analysis by GC were also collected downstream from the dam at the confluence of flows from the fish ladders and spillway. When hydrocarbons were present in the west fish ladder the concentration in midstream was 0.7 mg/L or less. Thus, all fish approaching the dam during a test period may have encountered aromatic hydrocarbons downstream.

Discussion

Mature salmon migrating upstream during the peak of the run did not distinctly avoid (AL50) a mixture of hydrocarbons at concentrations less than 3.2 mg/L. This concentration is bracketed by the concentrations of aromatic hydrocarbons avoided by juvenile coho salmon tested under laboratory conditions; AL50 values were 1.9 and 3.7 mg/L for smolt and presmolt coho, respectively (Maynard and Weber 1981). The latter studies were conducted in freshwater and whether or not similar relationships between hydrocarbon concentration and avoidance hold for higher salinity waters is not known. Movement of adult salmon into the fish ladders at Chambers Creek occurs predominantly at high tide and at high tide only the top one-half of the fish ladders is exposed. Thus, the tests with mature salmon represent avoidance of hydrocarbons under estuarine rather than strictly freshwater conditions.

It should be emphasized that the AL50 values for migrating adult salmon are estimates. When aromatic hydrocarbons were present in the west ladder, the salmon had the alternatives of (1) migrating up the hydrocarbon-contaminated west ladder, (2) migrating up the uncontaminated east ladder, or (3) remaining below the dam. If a relatively higher percentage of fish passed up the east ladder during experimental than during control periods, the result would be an apparent greater avoidance of the hydrocarbon than would be the case if most of those fish "destined" for the west ladder remained below the dam. There are no data available to evaluate this because no feasible method for accurately making such determinations was devised.

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Which Witch is Which? A Study of the Stock Structure of Witch Flounder (*Glyptocephalus cynoglossus*) in the Newfoundland Region

D. J. FAIRBAIRN[1]

Research and Resource Services, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre,
P.O. Box 5667, St. John's, Nfld. A1C 5X1

FAIRBAIRN, D. J. 1981. Which witch is which? A study of the stock structure of witch flounder (*Glyptocephalus cynoglossus*) in the Newfoundland region. Can. J. Fish. Aquat. Sci. 38: 782–794.

Genetic variability at two protein loci (phosphoglucomutase and superoxide dismutase) was assessed both within and among three major management areas for witch flounder (*Glyptocephalus cynoglossus*) in the Newfoundland region. The genetic analysis revealed population subdivision within each of the major management areas, giving a total of six subpopulations or stocks. These genetically defined stocks also differed with respect to population structure, time of spawning, individual growth rate, and temperature and depth of capture. These results suggest that witch flounder in the Newfoundland region exist in relatively small, independent subpopulations with little inter-stock migration. This population structure is at odds with current practices of managing witch flounder as large homogeneous stock units.

Key words: witch flounder, stock discrimination, biochemical genetics, phosphoglucomutase, superoxide dismutase, electrophoresis

FAIRBAIRN, D. J. 1981. Which witch is which? A study of the stock structure of witch flounder (*Glyptocephalus cynoglossus*) in the Newfoundland region. Can. J. Fish. Aquat. Sci. 38: 782–794.

L'auteur évalue la variabilité génétique à deux loci protéiques (phosphoglucomutase et superoxyde dismutase) tant intra qu'interrégionale dans les trois principales zones de gestion de la plie grise (*Glyptocephalus cynoglossus*) à Terre-Neuve. L'analyse génétique démontre que, dans chacune de ces zones, la population se subdivise, ce qui donne un total de six sous-populations ou stocks. Ces stocks génétiquement définis diffèrent également par la structure, le temps de la ponte, le taux de croissance individuel, et la température et la profondeur de capture. Ces résultats laissent supposer que la plie grise de la région de Terre-Neuve existe en sous-populations indépendantes, relativement petites, avec très peu de migration d'un stock à l'autre. Cette structure de population est à l'encontre des pratiques courantes selon lesquelles l'espèce est gérée comme grandes unités homogènes.

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WITCH flounder (*Glypocephalus cynoglossus*) is a flatfish of the family Pleuronectidae. It is distributed in the western north Atlantic from North Carolina to Labrador (Markle 1975; Bowering 1976a) and in the Newfoundland region is associated with muddy bottoms at depths greater than 140 m (Pinhorn 1976; Bowering 1976b). During recent years it has become an important commercial species in Newfoundland waters (Bowering and Pitt 1978; Bowering 1976a), although much of the fishery remains a by-catch of other groundfish species. The offshore fishery for witch in the Newfoundland region is currently regulated as four independent management areas or stocks. The most northern of these areas encompasses the Northwest Atlantic Fisheries Organization (NAFO) Divisions 2J, 3K, and 3L (Fig. 1) and may be termed the northeast Newfoundland and northern Grand Bank stock. This is the major commercial offshore stock (Bowering 1976b, 1978a). Witch found along the southern slopes of the Grand Bank (NAFO Div. 3N and 3O) are considered a separate stock (Bowering 1978b, 1979b). The two remaining offshore stocks of witch are found on the St. Pierre Bank (NAFO Div. 3Ps) and in the Gulf of St. Lawerence (NAFO Div. 3PN, 4RS).

The division of witch into these four offshore management units or stocks was initially based upon the distribution of prespawning and spawning concentrations of witch flounder observed in the winter and spring (W. R. Bowering, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Nfld., personal communication). A more recent study has indicated some variability among these areas in growth rates

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[1] Current address: 104 Westcliffe Avenue, Pointe Claire, Que. H9R 1M6.

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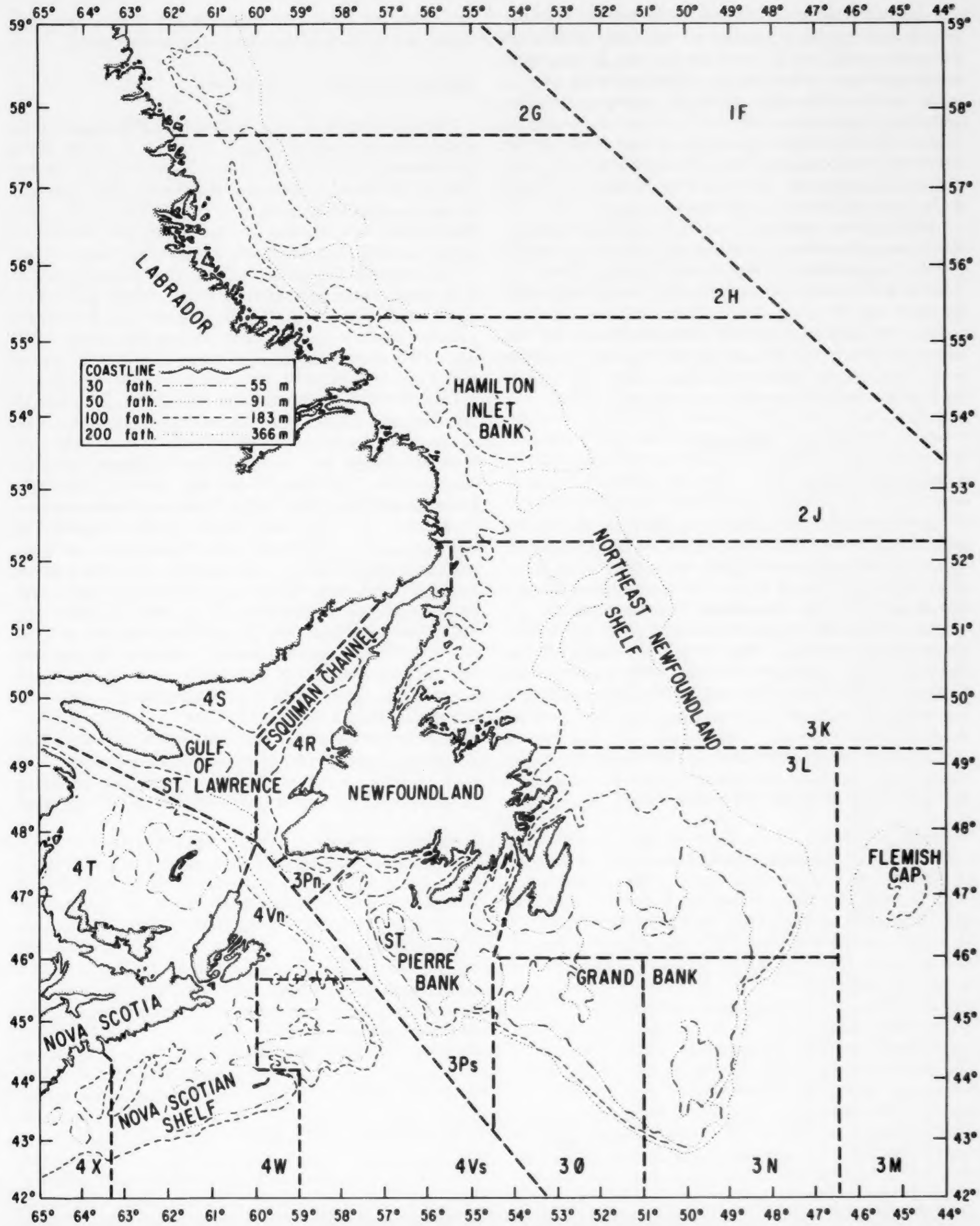
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2G
1F
LABRADOR
2H
COASTLINE
30 fath. 55 m
50 fath. 91 m
100 fath. 183 m
200 fath. 366 m
HAMILTON INLET BANK
2J
NORTHEAST NEWFOUNDLAND SHELF
ESQUIMAN CHANNEL
4S
GULF OF ST. LAWRENCE
4R
3K
3L
NEWFOUNDLAND
FLEMISH CAP
4T
3Pn
4Vn
ST. PIERRE BANK
GRAND BANK
NOVA SCOTIA
NOVA SCOTIAN SHELF
3Ps
4X
4W
4Vs
3O
3N
3M

FIG. 1. Place names and NAFO Divisions referred to in the text.

and age and length at maturity (Bowering 1976a). However, a close correspondence between the variability in these life history parameters and the four stock areas has not been firmly established: many of the differences between stock areas are slight and not statistically significant, and a closer examination of growth rates within the northern stock area has revealed heterogeneity within this area (Bowering and Pitt 1978). Thus, there remains little evidence that the stock areas used for management reflect the true structuring of witch flounder populations in the northwest Atlantic.

This study was undertaken with the aim of determining if these management stocks do indeed correspond to genetically isolated subpopulations. The overall hypothesis was that witch in the northwest Atlantic exist as several independent breeding units or subpopulations whose distributions correspond to the stock areas used for management purposes. This hypothesis was tested by examination of genetic variability within and among these stock areas using two electrophoretically detectable protein loci as genetic markers.

Methods

SAMPLE COLLECTION

Samples of witch were collected in the spring of 1979 and the winter of 1980, during the period of the prespawning and spawning concentrations of stocks. The sample areas, dates, and numbers are given in Table 1. All samples were collected by personnel of the Groundfish Program, Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland, Canada. Three of the four major offshore stock areas were sampled for a total of 464 witch from the northeast Newfoundland and northern Grand Bank area, 154 witch from the southern Grand Bank area, and 200 witch from the Gulf of St. Lawrence. Total length, sex, and stage of sexual maturity were recorded for each individual. Samples of muscle, liver, and heart were sealed in plastic bags and frozen at −20°C until analyzed, and otoliths were returned to the laboratory for aging (Bowering 1976a). The temperature, depth, and location of each haul were also recorded.

BIOCHEMICAL GENETIC TECHNIQUES

Isozyme phenotypes were assayed by horizontal starch gel electrophoresis using gels made up of 12.5% potato starch (Electrostarch batch No. 307, or Connaught Starch lot No. 350-1). The tissue samples were prepared for electrophoresis by homogenization in an equal volume of 0.9% saline. Filter paper wicks were inserted into the homogenate, blotted on paper towelling, and then inserted into a slit in the starch gel. A discontinuous Tris–citrate buffer system (electrode buffer: 0.30 mol/L borate, pH 8.2; gel buffer: 0.076 mol/L Tris, 0.005 mol/L citric acid, pH 8.7) (Selander et al. 1971) gave excellent results for most proteins. Several loci, notably MDH and LDH, were better resolved using a continuous bisTris buffer (electrode buffer: 8.41 g citric acid, 52.97 g bis Tris/L; gel buffer: 50 mL electrode buffer /L) (H. Tsuyuki, Fisheries and Oceans Canada, Food Technology Laboratory, Vancouver, B.C., personal communication).

Fifteen protein loci[2] were resolved in samples of witch muscle, liver, and heart, but only two loci, phosphoglucomutase (PGM) (E.C. 2.7.5.1) and superoxide dismutase (SOD) (E.C. 1.15.1.1) were found to be polymorphic. The latter appeared as white bands on all gels stained for dehydrogenase activity and on gels stained for PGM, but was best resolved using a stain composed of 150 mL 0.2 mol/L Tris-HCl (pH 8.0), 30 mg PMS, and 45 mg MTT (Selander et al. 1971). The white bands appeared after incubation at 37°C for ~½ h. SOD gave strongest activity in the liver and was very faint in heart samples. It appeared in all tissues as single-banded homozygotes, and triple-banded heterozygotes, reflecting its dimeric structure.

PGM was resolved after incubation at 37°C for ~15 min in a stain composed of 15 mL 0.2 mol/L Tris pH 8.0, 75 mL H_2O, 15 mL 0.1 mol/L $MgCl_2$, 60 units glucose-6-phosphate dehydrogenase, 15 mg NADP, 6 mg PMS, 15 mg MTT,

TABLE 1. Witch samples collected in 1979/80.

Vessel	Trip No.	NAFO area	Stock area	Sample dates	No.
Gadus Atlantica	18	3KL	Northeast Nfld. and northern Grand Bank	Mar. 9–11 1979	112
Gadus Atlantica	21	3L	Northeast Nfld. and northern Grand Bank	May 17–23 1979	199
A. T. Cameron	290	3L	Northeast Nfld. and northern Grand Bank	May 17–18 1979	153
A. T. Cameron	289	3N	Southern Grand Bank	Apr. 29–May 4 1979	92
A. T. Cameron	291	3O	Southern Grand Bank	June 13–21 1979	62
Gadus Atlantica	31	3Pn 4RS	Gulf of St. Lawrence	Jan. 27–Feb. 1 1980	200

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[2] Superoxide dismutase (SOD), phosphoglucomutase (PGM), phosphohexose isomerase (PHI-1 and PHI-2), aspartate amino transferase (AAT), malate dehydrogenase (MDH-1 and MDH-2), malic enzyme, lactate dehydrogenase (LDH), xanthine dehydrogenase (XDH), isocitrate dehydrogenase (ICDH-1 and ICDH-2), glucose-6-phosphate dehydrogenase (G-6-PDH), glutamate dehydrogenase (GDH), succinate dehydrogenase (SDH).

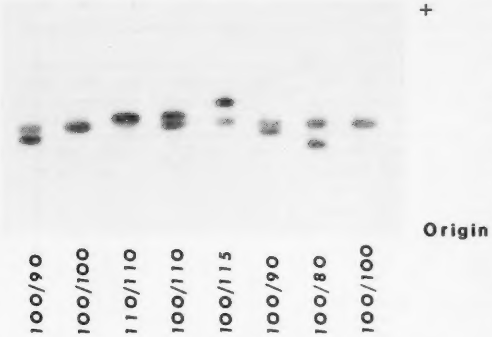

FIG. 2. PGM phenotypes from witch muscle.

15 mL 0.05 mol/L glucose-1-phosphate (Selander et al. 1971). The primary locus was slightly cathodal to SOD, but prolonged staining in muscle revealed a second very faint area of activity anodal to the SOD activity. The second locus could not be read reliably and was not used. The primary locus appeared as single-banded homozygotes and double-banded heterozygotes (Fig. 2). Repeated freezing and thawing of samples, or prolonged sample storage at −20°C (more than 4 mo) tended to produce secondary, anodal banding on PGM gels. Similar bands occurred if samples were frozen or thawed slowly. PGM is known to be particularly prone to secondary isozyme formation (Fisher and Harris 1972; Dawson and Greene 1975; Turner et al. 1975; Beckman 1978). For this reason, PGM was assayed only in new samples. No such secondary changes were evident on SOD gels.

Alleles at the PGM and SOD loci were classified according to their mobility relative to the most common allele which was designated '100' (Allendorf and Utter 1979).

TESTING THE GENETIC MODELS OF ISOZYME VARIABILITY

When using electrophoretically detectable protein loci (isozymes) as genetic markers, it is necessary to ascertain that the isozyme variability observed does reflect genetic variability, and, more specifically, that the inheritance of the observed isozyme patterns follows a specified genetic model (Brooke 1964; deLigny 1969; Utter et al. 1974; Allendorf and Utter 1979; Fairbairn and Roff 1980). Because it was not possible to breed and rear witch flounder in the laboratory, it was necessary to test this hypothesis indirectly. To do this I followed the methods suggested in Fairbairn and Roff (1980) and Utter et al. (1974). The isozyme phenotypes were observed in a large number of individuals (n = 818) and no unexpected phenotypes were found. The genetic models, developed from the observed isozyme patterns, corresponded to those reported elsewhere for PGM (Utter and Hodgins 1970; Altukov et al. 1975; Place and Powers 1978; Smith and Jamieson 1978; Kimura 1978; Odense et al. 1978) and SOD (Utter 1971; Utter et al. 1973; Iwata 1975; Johnson 1977; Smith and Jamieson 1978). Further, these patterns did not vary from tissue to tissue, and could not be generated by storage or changes in methods of tissue preparation. To check these genetic models further, the data from each cruise were analyzed for the effect of sex, degree of maturity, age, temperature, and depth on isozyme phenotype and allele frequency. In no case was any of these effects significant.

Next, the observed phenotypic distributions were compared with those expected according to the Hardy–Weinberg law. Significant deviations from Hardy–Weinberg expectations could indicate errors in the genetic models or deviations within the samples from the assumptions of panmixia or neutrality. Five of the six cruise samples showed good agreement with Hardy–Weinberg expectations at the PGM locus, and all agreed with Hardy-Weinberg expectations at the SOD locus (Tables 2 and 3). This is further evidence that the genetic models are correct. However, a significant deficiency of PGM heterozygotes, and in particular, *PGM*-100/*PGM*-110 heterozygotes, was evident in the Gadus 31 sample. A comparison of observed and expected frequencies revealed that the deficiency of heterozygotes was evident only in females (males: χ^2 = 0.026, df 1, $P > 0.50$; females: χ^2 = 11.105, df 1, $P < 0.001$). The females in this sample also exhibited two alleles (*PGM*-105 and *PGM*-125) that were not found in males or in any of the other cruise samples. Because of the presence of these unique alleles and of a significant heterozygote deficiency, I suspected an error in the genetic model due to misclassification of these alleles. Therefore, all Gulf samples were rerun to check the classification of phenotypes. No errors were found. The presence of the rare alleles was also not associated with the maturity, age, or length of the females. Grouping these alleles with other alleles of similar mobility did not remove the heterozygote deficiency. I therefore concluded that the observed heterozygote deficiency in females and the presence of unique alleles indicated violations of the assumptions of panmixia and/or neutrality for the Gulf of St. Lawrence sample, rather than errors in the genetic model for PGM.

Results and Discussion

GENETIC VARIATION WITHIN MANAGEMENT AREAS

The Gulf of St. Lawrence — To test the hypothesis of population subdivision within the Gulf suggested above by the initial comparisons of observed and expected phenotypic distribution, the Gulf sample was subdivided into unit areas, ½° latitude by 1° longitude (Fig. 3) and the phenotypic distributions were examined within each of these units. An examination of Table 4 reveals that the unit areas tend to fall into two groups. In units G26, G25, and H24, the frequency of *PGM*-100 is high in males and relatively low in females, and males show a slight excess of heterozygotes while females show a deficiency. Neither rare allele is present. Units J27 and J28 differ from this group in all these aspects while units H27 and H25 are intermediate between these two groups. For further analysis, the latter was included with G26, G25, and H24 because of its location. The former was included with J27 and J28 because of the low frequency of *PGM*-100 in males and the lack of a heterozygote deficiency in females. Its location was intermediate between the other groups. The samples from units G26, G25, H25, and H24 were taken from the Esquiman Channel in the northeastern area of the Gulf of St. Lawrence (Fig. 1 and 3) while the remaining samples were clustered fairly close to the southwestern shore of New-

TABLE. 2. Observed and expected[a] phenotypic distributions by trip for phosphoglucomutase.

	Trip (NAFO Division)											
	G18 (3KL)		G21 (3L)		ATC 290 (3L)		ATC 289 (3N)		ATC 291 (3O)		G31 (3Pn, 4RS)	
Phenotype	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
100/100	71	70.72	154	152.14	115	113.02	64	62.78	45	46.17	148	145.35
100/110	27	28.61	38	41.10	29	32.66	20	21.48	12	10.35	29	34.95
100/115	5	4.77									4	5.11
100/120	3	2.38	0	0.87	0	0.86	0	1.65	1	0.86	1	0.85
100/70	1	0.79	1	0.87	2	1.72						
100/85							4	3.30			1	0.85
100/90					2	1.72			2	1.73	5	4.26
100/80			1	0.87					2	1.73		
100/105											4	3.41
100/125											1	0.85
110/110	4	2.89	4	2.78	4	2.36	2	1.84	0	0.58	6	2.10
110/115	1	0.96									0	0.61
110/120	0	0.48	1	0.12	1	0.12	2	0.28	0	0.10	0	0.10
110/70	0	0.16	0	0.12	0	0.25						
110/85							0	0.57			0	0.10
110/90					0	0.25			0	0.19	0	0.51
110/89			0	0.12					0	0.19		
110/105											0	0.41
110/125											0	0.10
115/115	0	0.08									1	0.04
Rare phenotypes[b]	0	0.14			0	0.09	0	0.05	0	0.11	0	0.32
χ^2[c]	1.043		0.792		1.613		0.142		1.570		8.325	
df	2		1		1		1		1		1	
P	>0.50		>0.30		>0.10		>0.50		>0.20		<0.005	

[a] Based on the Hardy–Weinberg distribution.

[b] Phenotypes whose expected value is always <0.01, and whose observed value always = 0.

[c] Alleles other than 100 and 110 grouped as rare alleles. Rare phenotypes grouped as required.

TABLE. 3. Observed and expected[a] phenotypic distributions by trip for superoxide dismutase.

	Trip (NAFO Division)											
	G18 (3KL)		G21 (3L)		ATC 290 (3L)		ATC 289 (3N)		ATC 291 (3O)		G31 (3Pn, 4RS)	
Phenotype	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
100/100	105	105.11	184	184.28	138	138.37	92	92.00	54	54.26	187	187.24
100/70	7	6.78	12	11.55	15	14.26			7	6.55	10	9.65
100/40			3	2.89					1	0.94	3	2.90
100/132											1	0.97
70/70	0	0.11	0	0.18	0	0.37			0	0.20	0	0.12
70/40			0	0.09					0	0.06	0	0.07
70/132											0	0.02
40/40			0	0.01							0	0.01
40/132											0	0.01

[a] Based on the Hardy–Weinberg distribution.

foundland. The differences between these groups become obvious when the relevant unit areas are combined. The deficiency of heterozygotes is significant for the Esquiman group for both the total sample ($F = 0.280$, $\chi^2 = 9.722$, df 1, $P < 0.005$) and for females alone ($F = 0.356$, $\chi^2 = 10.781$, df 1, $P < 0.005$). Males show no significant deviation from Hardy–Weinberg expectation. The distribution of penotypes differs significantly between males and females in this sample ($G = 4.345$, df 1, $P < 0.05$), males having a significantly higher frequency of *PGM*-100.

Because the heterozygote deficiency in Esquiman Channel group occurs down to the unit area level, it is unlikely to reflect population subdivision. The remaining hypothesis is sex-specific selection. If we assume that the phenotypic frequencies in males and females were equal at conception, and that the sampled male phenotypic distribution reflects this

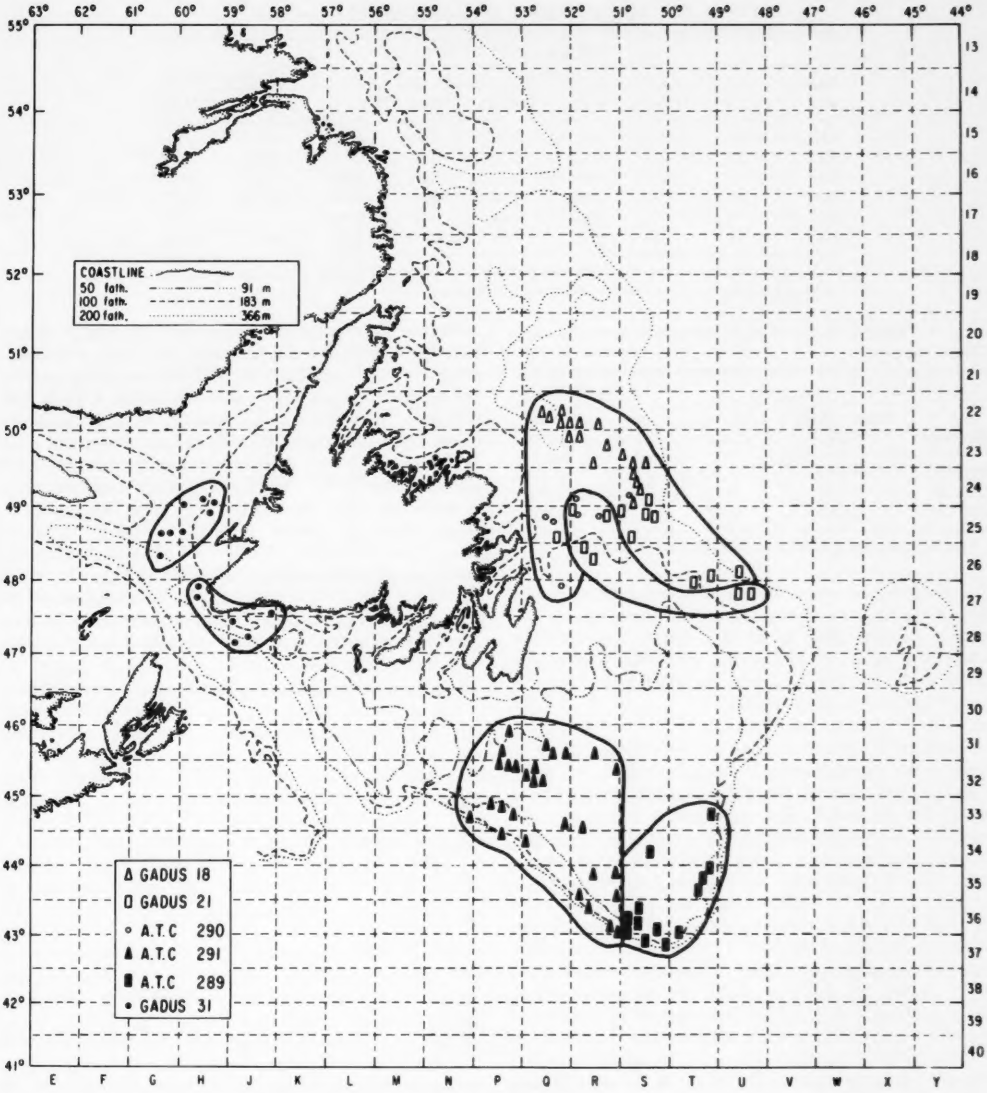
63° 62° 61° 60° 59° 58° 57° 56° 55° 54° 53° 52° 51° 50° 49° 48° 47° 46° 45° 44°
55° 54° 53° 52° 51° 50° 49° 48° 47° 46° 45° 44° 43° 42° 41°
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
E F G H J K L M N P Q R S T U V W X Y
COASTLINE
50 fath. 91 m
100 fath. 183 m
200 fath. 366 m
△ GADUS 18
▯ GADUS 21
○ A.T.C 290
▲ A.T.C 291
■ A.T.C 289
● GADUS 31

FIG. 3. Locations of sample sets for the six cruises. Solid lines separate witch stocks detected by genetic analysis. Dotted lines separate unit areas.

original distribution (since it follows Hardy–Weinberg expectations very closely), we can very crudely estimate the relative fitnesses of the female phenotypes as in Table 5. This analysis suggests extremely strong selection favoring rare phenotypes in females. The presence of PGM alleles 115, 85, and 90 in females but not males, and the lower frequency of *PGM*-100 in females further suggest selection for rare alleles in females.

The samples from southwest Newfoundland do not show a difference between males and females (G = 1.247, df 2, $P > 0.50$) or between observed and expected distributions (total: $\chi^2 = 0.964$, df 1, $P > 0.30$; males: $\chi^2 = 0.109$, df 1, $P > 0.50$; females: $\chi^2 = 0.145$, df 1, $P > 0.50$). Rare alleles, *PGM*-115 and *PGM*-90, occur in both males and females, and the frequency of *PGM*-100 does not differ between sexes (males: 0.824, females: 0.847). Alleles *PGM*-105 and *PGM*-

TABLE 4. Analysis of Gulf of St. Lawrence samples by unit area.

Unit area	*N*		Frequency of *PGM*-100		F^a		Presence of:	
	Males	Females	Males	Females	Males	Females	*PGM*-105	*PGM*-125
J27/28	27	35	0.85	0.90	0.16	−0.08	√	√
H27	10	24	0.75	0.77	−0.33	0.00	—	—
G26	12	17	0.96	0.77	−0.04	0.09	—	—
G25	16	14	0.94	0.78	−0.06	0.59	—	—
H25	8	9	0.94	0.94	−0.06	−0.06	—	—
H24	12	14	0.92	0.75	−0.09	0.48	—	—

[a] $F = 1 - (H_o/H_e)$ where H_o is the observed number of heterozygotes, and H_e is the number expected according to the Hardy–Weinberg distribution.

TABLE 5. Relative fitness of PGM phenotypes in the Esquiman Channel sample.

Phenotype	Frequency Before selection (males)	Frequency After selection (females)	Ratio	'Relative fitness'
100/100	0.879	0.685	0.779	0.034
100/110[a]	0.117	0.222	1.897	0.082
110[a]/110[a]	0.004	0.093	23.25	1.000

[a] One hundred and ten includes all alleles other than 100.

125, however, are unique to this area and occur in females only. It is perhaps significant that females from this area also possess a unique allele at the SOD locus, *SOD*-132. The presence of these rare alleles only in females may be due merely to the fact that the sample contained almost twice as many females (59) as males (37).

Northeast Newfoundland and northern Grand Bank — Three of the cruises (Gadus Atlantica 18 and 21, and A. T. Cameron 290) sampled this northern stock area, covering NAFO Div. 3K and 3L. The most northern part of the stock area, Div. 2J, was not sampled. The distribution of samples by trip is illustrated in Fig. 3. The distribution of PGM phenotypes differed significantly among these three cruises (G = 13.065, df 6, $P > 0.05$), as did the allele frequencies ($G = 21.172$, df 6, $P < 0.005$) (Table 6). However, the two 3L samples (Gadus 21 and A. T. Cameron 290) did not differ from each other in either phenotypic distributions (G = 1.363, df 3, $P > 0.50$) or allele frequencies ($G = 1.266$, df 2, $P > 0.50$). These two samples combined did differ significantly from the 3KL samples (Gadus 18) in both phenotypic frequencies ($G = 11.703$, df 3, $P < 0.01$) and allele frequencies ($G = 19.742$, df 3, $P < 0.005$). The more northern sample showed a lower frequency of *PGM*-100 than the 3L samples and also possessed a rare allele, *PGM*-115, which was not found further south. Because there is some overlap between the areas sampled by the 3L and 3KL trips (Fig. 3), it is unlikely that the difference between these two samples accurately reflects the distribution of subpopulations or stocks within this stock area. To assess more accurately the distribution of subpopulations, the frequency of the *PGM*-100 and *PGM*-115 alleles was examined by unit area. Where a given unit was sampled by more than one trip, *PGM*-100 frequencies were compared. In these cases, no difference was ever found between trips. The data for all trips were thus combined within each unit. Only units with sample sizes >5 (alleles sampled >10) were included in the analysis. The results of the analysis are presented in Fig. 4. Units R24, R25, R26, and U27 can be distinguished by a high frequency of the *PGM*-100 allele (≥0.875) and the absence of allele *PGM*-115. In all other units, the frequency of the *PGM*-100 allele is below 0.830. The *PGM*-115 allele is present in four of the seven units. The difference in allele frequencies between these two groups of units is highly significant ($G = 15.422$, df 2, $P < 0.005$). All of the high frequency units were sampled by one or both of the 3L trips. They differ significantly from the remaining units sampled during those trips ($G = 9.664$, df 2, $P < 0.01$) and also from the units sampled during the 3KL trip ($G = 13.016$, df 2, $P < 0.005$).

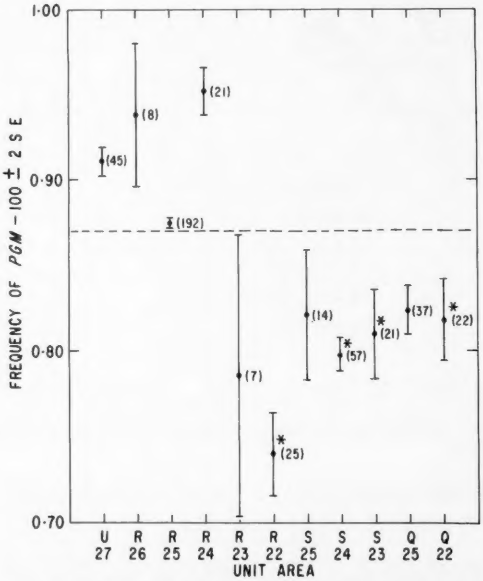

FIG. 4. Frequency of *PGM*-100 (mean ± 2 SE) by unit area for NAFO Div. 3K and 3L. Sample sizes are in parenthesis. *Indicates the presence of allele *PGM*-115.

TABLE 6. Allele frequencies at the phosphoglucomutase locus, by trip.

	Cruise (NAFO Division)					
Allele	G18(3KL)	G21(3L)	ATC 290(3L)	ATC 289(3N)	ATC 291(3O)	G31 (3Pn4RS)
100	0.795	0.874	0.859	0.826	0.863	0.852
110	0.161	0.118	0.124	0.141	0.097	0.102
115	0.027					0.015
120	0.013	0.003	0.003	0.011	0.008	0.002
70	0.004	0.003	0.007			
85				0.022		0.002
90			0.007		0.016	0.012
80		0.003			0.016	
105						0.010
125						0.002

TABLE 7. Allele frequencies at the superoxide dismutase locus, by trip.

	Cruise (NAFO Division)					
Allele	G18(3KL)	G21(3L)	ATC 290(3L)	ATC 289(3N)	ATC 291(3O)	G31 (3Pn4RS)
100	0.969	0.962	0.951	1.000	0.935	0.965
70	0.031	0.030	0.049		0.056	0.025
40		0.008			0.008	0.007
132						0.002

The 3KL units do not, however, differ from the low frequency 3L units ($G = 4.131$, df 2, $P > 0.10$).

It is likely that this division of samples, although still crude, more accurately reflects the subdivision of populations within the northern stock area than would a division based only on the differences observed between trips. Henceforth the low *PGM*-100 frequency subpopulation will be termed the northeast Newfoundland sample, and the high frequency *PGM*-100 group will be called the northern Grand Bank sample. The distribution of these groups suggests overlap between their ranges (Fig. 3). The apparent genetic isolation must therefore arise from differences in spawning location or timing.

No comparable differences at the SOD locus were found between these PGM-defined samples ($G = 1.48$, df 2) or between the original 3KL and 3L trips ($G = 0.781$, df 2) (Table 7).

Southern Grand Bank — The two samples from this stock area (3N and 3O) did not differ in either phenotype frequencies ($G = 0.049$, df 1, $P > 0.50$) or allele frequencies ($G = 1.716$, df 3, $P > 0.50$) at the PGM locus (Tables 2 and 6). However, the frequency of the *SOD*-100 allele was significantly higher in the 3N than in the 3O sample ($G = 10.676$, df 1, $P < 0.005$) (Table 7). This suggests possible population subdivision within the southern Grand Bank stock area. Because of the small sample size ($n = 62$), the high frequency of *SOD*-100, and the large area covered by the 3O sample (Fig. 3), it is not possible to subdivide the sample into unit areas to analyze the distribution of rare alleles. (Exclusion of the rare alleles from such subsamples would more likely be due to sample size than absence from that region.) Samples of sufficient size could, however, be obtained by grouping some contiguous units. Analysis of these groups (Table 8) revealed that the rare allele (*SOD*-70) is distributed throughout at least the central and northwestern parts of the sample area. The tendency, if any, is for rare allele frequencies to increase toward the southeast (toward NAFO Div. 3N). Therefore, our best estimate of the true subdivision of stocks within the southern Grand Bank area remains that dictated by the cruise samples: henceforth, Div. 3O and 3N shall be considered separate stock areas.

TABLE 8. Frequencies (2 SE) of *SOD*-100 in Division 3O.

Unit area	Frequency of *SOD*-100 (2 SE)	*n*
P 31/32	0.962 (0.010)	13
P 33/34	0.955 (0.013)	11
Q 31	0.921 (0.010)	19
Q 32/33	0.875 (0.019)	12

GENETIC VARIATION AMONG AREAS

The foregoing analyses have suggested genetic heterogeneity within each of the three management areas. This precludes the treatment of each management area as a single stock, and suggests that that aspect of our initial hypothesis is incorrect. Although the detailed analyses of the pattern of genetic variation within each area were necessarily crude, they suggested the presence of at least two subpopulations or stocks within each of the management areas. Therefore, comparisons among areas now involve six stocks, rather than three. The allele frequencies and sample sizes for these six stocks are seen in Tables 9 and 10. The PGM allele frequencies vary significantly with area. The preceding analyses have shown that the two northern areas differ significantly at this locus, but that the two southern Grand Bank areas do not. The two Gulf areas also do not differ in allele frequency when males and females are combined ($G = 4.487$, df 4, $P > 0.30$). Therefore, for further comparisons of PGM allele

TABLE 9. PGM allele frequencies by subpopulation or stock.

	Stock					
			Southern Grand Bank			
Allele	Northeast Nfld.	Northern Grand Bank	3N	30	Southwest Nfld.	Esquiman Channel
100	0.797	0.889	0.826	0.863	0.840	0.864
110	0.172	0.100	0.141	0.097	0.103	0.102
115	0.015	—	—	—	0.015	0.015
120	0.010	0.002	0.011	0.008	0.005	—
70	0.003	0.006	—	—	—	—
85	—	—	0.022	—	—	0.005
90	0.003	0.002	—	0.016	0.10	0.015
80	—	0.002	—	0.016	—	—
105	—	—	—	—	0.021	—
125	—	—	—	—	0.005	—
n	195	266	92	62	97	103

Comparison	*G*	df	*P*
Total	26.641	10	<0.005
Northeast Nfld. × northern Grand Bank	15.422	2	<0.0005
Northeast Nfld. × southern Grand Bank	3.258	2	NS
Northeast Nfld. × Gulf	8.781	2	<0.05
Northern Grand Bank × southern Grand Bank	6.992	2	<0.05
Northern Grand Bank × Gulf	10.539	2	<0.01
Southern Grand Bank × Gulf	1.070	2	NS

TABLE 10. SOD allele frequencies by subpopulation or stock.

	Stock					
			Southern Grand Bank			
Allele	Northeast Nfld.	Northern Grand Bank	3N	30	Southwest Nfld.	Esquiman Channel
100	0.956	0.962	1.000	0.935	0.959	0.971
70	0.038	0.036	—	0.056	0.026	0.024
40	0.005	0.002	—	0.008	0.010	0.005
132	—	—	—	—	0.005	—
n	195	266	92	62	97	103

Comparison	χ^2	df	*P*
Total	10.830	5	<0.10
3O × northern areas	1.042	1	NS
3O × Gulf areas	3.046	2	NS
3N × northern areas	6.449	1	<0.025
3N × Gulf areas	5.156	1	<0.025

frequencies, both the two Gulf samples and the two southern Grand Bank samples are pooled. The relative similarities of these pooled stocks and the two northern stocks can be determined from the comparisons in Table 9. The greatest difference occurs between the northern Grand Bank and northeast Newfoundland samples. The next largest differences are seen between these areas and the Gulf. The southern Grand Bank shows the least difference from all other areas, and differs significantly only from the northern Grand Bank.

The SOD locus shows little variation among areas. The preceding analyses have shown no significant differences between the northern or the Gulf stocks. However, the southern Grand Bank stocks do differ significantly and area 3N also differs significantly from the Gulf and from the northern stocks (Table 10), although no such differences are apparent for area 3O. Thus, at the SOD locus, area 3N differs from all others, but no other pattern of variation can be discerned.

DISTRIBUTION BY DEPTH AND TEMPERATURE

The general hydrology of the shelf and slope waters of the Newfoundland region has been examined in detail elsewhere (Templeman 1966; Burmakin 1972; Kudlo 1975), as has the general distribution of witch flounder by depth and temperature (Bowering 1976a). The data gathered for this study are insufficent for such a general review, but may suggest habitat differences among the six stocks. Both water temperature and depth of capture varied significantly among the

TABLE 11. Mean depth of capture and bottom temperature by area.

Area	Mean depth (m)	Mean temp. (°C)
Northeast Nfld.	295.4	2.8
Northern Grand Bank	300.4	2.8
Southern Grand Bank: 3N	216.9	4.9
Southern Grand Bank: 30	108.2	1.7
Gulf: Esquiman Channel	330.5	5.3
Gulf: Southwest Nfld.	380.5	5.6
F	195.5	217.10
df	5, 813	5, 813
P	<0.0001	<0.0001

six stock areas (Table 11). However, witch flounder from the two northern areas were captured under very similar conditions. These conditions are within the range of maximum abundance of witch throughout the northern region (Bowering 1976a) and reflect the common influence of the cold Labrador current (Templeman 1966; Burmakin 1972). Similarly, witch flounder from both Gulf areas were captured in the deeper, warmer water characteristic of distribution of witch within the Gulf (Bowering 1976a). Thus, there is little to suggest that the genetic differentiation within the Gulf or within the northern area reflects selective adaptations to local abiotic conditions. The two southern Grand Bank samples, however, do differ in both depth and temperature of capture. This apparent difference may be partly due to the summer migration of witch up onto the Grand Bank from the slope gullies (Markle 1975; Bowering 1976a): the 3O sample was taken later in the year than the 3N sample, and many of the witch captured in 3O were up in the cooler, shallower water on top of the Grand Bank. However, we might expect significant environmental differences between these two regions in any case since area 3N is under the more stable influence of the cool Labrador current, while area 3O is exposed to the more variable currents and temperatures of the slope water (Templeman 1966; Burmakin 1972). Thus, these two stocks may be specifically adapted to different abiotic conditions, although it is likely that similar conditions prevail in the southern area of their ranges, where the tentative stock boundary has been drawn.

COMPARISONS OF POPULATION CHARACTERISTICS

The six stock areas defined by the biochemical genetic analyses were compared with respect to the following population charcteristics: sex ratio, age distribution, length distribution, mean growth rate, and proportion mature. Summary statistics for these parameters are presented in Tables 12 and 13. Although the sex ratio varies significantly among areas ($G = 40.852$, df 5, $P < 0.005$), pairwise comparisons indicated that the only significant feature of the sex ratio distribution is the usually high proportion of males in the northern Grand Bank sample. The other samples are quite similar and approach the 50:50 sex ratio common in offshore stocks of witch (Bowering 1978a; Bowering and Pitt 1978).

The proportion of fish in various stages of sexual maturaity also varies significantly among the six stocks ($G = 273.75$, df 15, $P < 0.0005$). In this case, however, pairwise comparisons revealed highly significant differences ($P < 0.005$) between all areas except the two Gulf areas. Some of these differences may be due to differences in the time of sampling rather than to differences in spawning time or in the proportion of immature individuals in the population. To determine if the greater proportion of mature and spent individuals in the northern Grand Bank sample as compared to the northeastern Newfoundland sample was due merely to the inclusion of the early (March) Gadus Atlantica 18 witch in the latter sample, the northern Grand Bank sample was compared with only those northeast Newfoundland witch sampled during the A. T. Cameron 290 trip. The difference remained highly significant ($G = 27.916$, df 2, $P < 0.0005$), suggesting that the difference between the two northern samples is due at least partly to earlier spawning and fewer immature individuals in the northern Grand Bank stock.

A similar comparison can be made between the two southern Grand Bank samples. The higher proportion spent and lower proportion immature in the 3O sample may well be due merely to the fact that this sample was collected 6 weeks later than the 3N sample. The relatively low level of reproductive activity in the 3N sample when compared to all the other samples cannot, however, be accounted for by differences in sampling time, so must reflect true differences in the timing of spawning between these subpopulations. The southern populations, therefore, must spawn later than those further north.

Comparisons of lengths and ages among the six subpopulations can be seen in Table 13. Because female witch tend to be larger and to live longer than males (Bowers 1960; Halliday 1973; Bowering 1976a, 1978a; Bowering and Pitt 1978), the sexes were treated separately for these comparisons. Length varies signifiantly with area for both males ($F = 16.527$, df 5, 454, $P < 0.0001$) and females ($F = 15.411$, df 5, 360, $P < 0.0001$). Both sexes showed similar trends, being largest in the northern Grand Bank sample and smallest in the 3N sample. Bowering (1976a) concluded that the onset of sexual maturity in witch is a function of length, not age, and that length at onset of maturity varied little over the Newfoundland region. As would be expected according to this hypothesis, the small mean length in area 3N is reflected in a similarly low proportion mature. The opposite is true for the northern Grand Bank area which has the highest mean length and the highest proportion mature.

The overall regional differences in proportion mature observed in this study are in accordance with those reported by Bowering (1976a). He found a relatively low level of maturity in the Gulf of St. Lawrence, followed by the southwest Grand Bank (Area 3O). The northern areas (2J, 3KL) had the highest proportion mature. Although Bowering does not analyze variation within these major regions, the concordance of major trends between the two studies does indicate that between-stock variation in population structure is stable over time.

The population age structure also varies significantly among areas for both males ($\chi^2 = 79.690$, df 25, $P < 0.0005$) and females ($\chi^2 = 72.324$, df 25, $P < 0.0005$). For both sexes, the northeast Newfoundland sample has a lower mean age than the northern Grand Bank sample. This difference is in accordance with the lower mean length and lower proportion mature in the northeast Newfoundland sample. Similarly,

TABLE 12. Sex ratio and proportion mature for six subpopulations or stocks.

Stock	N	Proportion males	Proportion mature[a]			
			I	II	III	IV
Northeast Newfoundland	198	0.54	0.19	0.28	0.52	0.01
Northern Grand Bank	366	0.71	0.06	0.10	0.59	0.25
Southern Grand Bank: 3N	92	0.50	0.60	0.06	0.32	0.02
Southern Grand Bank: 30	62	0.56	0.18	0.19	0.37	0.26
Esquiman Channel	103	0.47	0.28	0.26	0.45	0.01
Southwest Newfoundland	98	0.38	0.44	0.23	0.32	0.01

[a] Maturity stages: I, immature; II and III, mature (active gonads); IV, spent (have completed spawning for this year).

TABLE 13. Comparisons of length, age, and growth among the six subpopulations or stocks.

	Mean length (cm)		Mean age (yr)		Length at 10 yr of age	
	Males	Females	Males	Females	Males	Females
Northeast Newfoundland	44.0	48.5	9.0	10.0	47.6	49.4
Northern Grand Bank	45.9	51.5	9.7	10.7	46.4	48.8
Southern Grand Bank: 3N	39.4	41.7	9.3	9.9	42.4	42.5
Southern Grand Bank: 30	44.1	44.6	10.2	10.4	43.4	43.8
Esquiman Channel	41.2	45.3	9.3	10.5	43.3	44.5
Southwest Newfoundland	42.7	45.2	10.1	11.0	42.7	43.2

the low proportion mature and small mean length in the 3N southern Grand Bank sample is reflected in a low mean age. Within the Gulf of St. Lawrence, the Esquiman Channel sample has both a lower mean age and a lower mean length than the southwest Newfoundland sample. The proportion mature is, however, higher (although not significantly so) in the Esquiman Channel sample. This may indicate a lower size/age at maturity in this sample.

Given these differences in length, age, and maturity, it is of interest to ask if growth rate also varies among the six stocks. For comparison of growth rates, length was regressed against the natural logarithm of age (Bowers 1960), and a covariance analysis was performed using multiple regression with area terms entered as dummy variables. The interaction effect was significant for both males ($F = 49.894$, df 5, 448, $P < 0.0005$) and females ($F = 8.125$, df 5,354, $P < 0.0005$), indicating that growth rate varied significantly among areas. The mean length predicted at age 10 (the modal age-class) by the regression analyses is presented in Table 13 as an index of comparative growth rates between sexes and areas. As expected, females grow faster than males in all areas (Bowers 1960; Bowering 1976a). Both sexes, however, show similar variation among areas. The fastest growth occurs in northeast Newfoundland, followed by the northern Grand Bank. Growth rates among the remaining areas are similar and low, with the slowest growth occurring in area 3N. This pattern of latitudinal variation in growth is similar to that reported by Bowering (1976a).

The overall concordance of these population characteristics with the genetic results, when comparisons are made either within or among the major stock areas, strongly supports the hypothesis that the population subdivisions suggested by the genetic analyses are real. For example, a comparison of PGM allele frequencies among all six stocks reveals the greatest contrast between the two adjacent northern stocks. These stocks differ not only from each other, but from the other areas as well, with the northern Grand Bank stock being the most divergent. This northern Grand Bank stock was also distinguished from all others by its unusually skewed sex ratio, high mean length, and high proportion mature. Similarly, the 3N southern Grand Bank stock is distinguished from all others in having only one allele at the SOD locus. This genetic isolation is paralleled by the unique population structure observed for this stock: it had significantly more small, young, immature individuals than any other stock. As a further example, analysis of both the protein loci, and the population parameters indicates that of the six subpopulations distinguished, those of Area 3O and the Gulf are the most similar.

POSSIBLE INFLUENCE OF FISHING MORTALITY

In the absence of interference by man, we might assume that the differences observed among the six subpopulations are the result of phenotypic and/or genetic adaptation to different environments, or reflect genetic drift following spatial or temporal separation of spawning groups. However, witch flounder is an exploited species, and it is possible that fishing mortality has influenced the patterns and degree of stock differentiation. Of the areas sampled for this study, only the northeast Newfoundland stock (NAFO Div. 3K and northern 3L) and the southern Grand Bank area support a significant fishery (Bowering 1976a; 1979a, b, c). Thus, fishing mortality cannot be responsible for the divergent character of the northern Grand Bank stock, which is south of the fishing grounds, or for the subdivision of the Gulf of St. Lawrence stocks which do not support a fishery. The fishery on the northeast Newfoundland stock began only in 1970, and has been declining since 1973 as a result of the diversion of effort to the cod and turbot fisheries (Bowering 1979b and c). It is now a relatively minor fishery, taking less than the total allowable catch, and having no detectable effect on the biomass or age distribution of witch in the area (Bowering 1979c). It is unlikely that the genetic structure and other population characteristics of this stock could have been significantly altered by a fishery of such low intensity and short duration.

A directed fishery for witch flounder along the southwest slope of the Grand Bank began in the late 1960's although witch had been captured as a by-catch of the haddock fishery before that time (Bowering 1979a, b). Although the effort directed toward this fishery is quite small, there is some indication of heavy fishing mortality, particularly on older age-classes and females (Bowering 1979a). It is difficult to assess the impact of this fishery in area 3N, since only the extreme southwestern portion of this area is included in the fishing grounds. However, the relatively low age, small size, and low genetic diversity at the SOD locus in the 3N stock would suggest that the impact, if any, has been higher here than in 3O, perhaps because of the small total population size of witch on the southeast slope (Bowering 1976a, 1979b). Thus, fishing mortality may have contributed to the divergence of the southern Grand Bank stocks from each other, and from the stocks in the other management areas.

Summary and Conclusions

The analysis of allele and genotype frequencies at the PGM and SOD loci indicated that the witch flounder population in the Newfoundland region is genetically heterogeneous. Witch were sampled from each of three major management areas for this species, and significant heterogeneity was found within as well as among these areas. Although only the more southern portions of the northern management area were sampled, analysis of PGM allele frequencies revealed two distinct subpopulations. Although these two subpopulations are not spatially isolated and appear to occupy similar environments, they differ in many respects. The northeast Newfoundland stock is composed of a higher proportion of smaller, younger, immature individuals, and a higher proportion of females, than the northern Grand Bank stock. Witch from the former area also grow faster and spawn later in the season. These data strongly support the hypothesis of at least two separate breeding stocks in this northern area.

Samples from the southern Grand Bank management area were homogeneous with respect to PGM allele frequency. However, witch from Div. 3N differed from those from Div. 3O and from all other populations in having only one allele at the SOD locus. The 3N stock was also distinguished by a high proportion of small, young, immature individuals. Witch from 3N also grew more slowly than those from other areas. Both the genetic and the phenotypic differences between these stocks may have arisen naturally as a result of the differences in environmental conditions between the southwestern and southeastern slopes of the Grand Bank. There is some indication, however, that the differentiation of these stocks, particularly the 3N stock, may have also been influenced by fishing mortality.

The third major stock area sampled was the Gulf of St. Lawrence. Genetic heterogeneity within this area was revealed by a significant deviation of the observed distribution of PGM phenotypes from that expected according to the Hardy–Weinberg law. This deviation was traced to a large deviation from that expected within females from the Esquiman Channel area of the Gulf. The distribution of PGM phenotypes and alleles differed significantly between males and females within this region. Both subsampling and comparison of the sexes suggested that the apparent heterozygote deficiency in females within this area was due to strong selection favoring rare phenotypes, rather than to the Wahlund effect. Neither the difference between the sexes, nor the deviation from that expected was evident in the remaining Gulf samples. These were characterized, however, by the presence of two unique PGM alleles and one unique SOD allele. The presence of these unique alleles suggests that this southwest Newfoundland area supports an isolated reproductive unit. Although spawning time did not differ between these two Gulf stocks, there were significantly fewer mature individuals in the southwest Newfoundland population. Since neither length nor growth rate differed significantly between areas, this difference in proportion mature may reflect a higher length or age at maturity in the southwest Newfoundland stock.

The fine-scale subdivision of witch flounder populations revealed by the preceding analyses is not unique to this species. Biochemical genetic studies have revealed subdivision on a similar scale in other demersal species such as cod (*Gadus morhua*) (Sick 1965; Møller 1966; Cross and Payne 1978; Jamieson and Turner 1978), walleye pollock (*Theragra chalcogramma*) (Iwata 1975), and whiting (*Merlanguis merlanguis*) (Wilkins 1971), as well as in herring (*Clupea harengus*) (Simonarson and Watts 1969; Ridgway et al. 1971) and various anadromous salmonids (Møller 1970; Payne et al. 1971; Utter et al. 1973; Altukhov et al. 1975). Such fine-scale subdivision must reflect very limited movement of witch between stocks. Studies of the morphometrics and meristics of witch flounder (W. R. Bowering, unpublished data), and limited observations of the movement of tagged adults (Bowering 1979b) provide further evidence that witch in the Newfoundland region exist in relatively small subpopulations or stocks with diverse life history characteristics and little inter-stock migration. Such a population structure is clearly at odds with the current management practice of treating large areas as unit stocks. The implications of this difference between the two stock concepts, particularly where fishing pressure is heavy, should not be overlooked.

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Temporal, Intra-population Differences in Energy Allocation and Use by American Shad (*Alosa sapidissima*) During the Spawning Migration[1]

B. D. GLEBE[2] AND W. C. LEGGETT

Department of Biology, McGill University, Montreal, Que. H3A 1B1

GLEBE, B. D., AND W. C. LEGGETT. 1981. Temporal, intra-population differences in energy allocation and use by American shad (*Alosa sapidissima*) during the spawning migration. Can. J. Fish. Aquat. Sci. 38: 795–805.

The intra-population tissue weight dynamics and bioenergetics of American shad (*Alosa sapidissima*) were studied during the freshwater migration in the Connecticut River. Emphasis was on the extent and significance of temporal differences in the magnitude and rate of depletion of stored body reserves. This was accomplished by the comparison of body reserves of shad sampled at various locations and times along the migratory route. Somatic weight loss and associated energy depletion was extreme (45–60%); up to 50 and 70% of available body protein and lipid, respectively, were used to fuel the migration. The extent of reserve depletion was sex- and size-specific. Small individuals and males suffered the greatest tissue depletion. High water temperatures accounted for the reduced energy efficiency of late migrant shad (average of 42 vs. 24 KJ · kg^{-1} · km^{-1} expended by earlier migrants), and resulted in, greater depletion of total body reserves. More extensive depletion may contribute to the higher mortality reported among this component of the population. Gonad development was complete prior to entry into the river. This may spare somatic reserves during the freshwater fast and hence maximize survival. The pattern and extent of energy utilization in other anadromous fish species are discussed with reference to possible influence on demographic parameters, especially the repeat spawning habit.

Key words: American shad, *Alosa sapidissima*; energetics, allocation, weight loss, temporal differences, Connecticut River, migration

GLEBE, B. D., AND W. C. LEGGETT. 1981. Temporal, intra-population differences in energy allocation and use by American shad (*Alosa sapidissima*) during the spawning migration. Can. J. Fish. Aquat. Sci. 38: 795–805.

On trouvera dans l'article qui suit les résultats d'une étude de la dynamique et de l'énergobiose du poids tissulaire au sein d'une population d'aloses savoureuses (*Alosa sapidissima*) durant leur migration en eau douce dans la rivière Connecticut. L'étude a porté surtout sur le degré et l'importance des différences temporelles dans l'étendue et le taux d'épuisement des réserves emmagasinées dans le corps. On y est arrivé en comparant les réserves corporelles d'aloses échantillonnées à divers endroits et dates le long de la route de migration. La perte de poids somatique et l'épuisement énergétique qui lui est associé sont extrêmement élevés (45–60%). La migration requiert jusqu'à 50 et 70% des protéines et des lipides corporels, respectivement. Le degré d'épuisement des réserves est spécifique au sexe et à la taille. L'épuisement tissulaire est le plus prononcé chez les petits sujets et les mâles. Chez les aloses dont la migration est tardive, des températures de l'eau élevées causèrent un amoindrissement de l'efficacité énergétique (moyenne de 42 vs. 24 KJ · kg^{-1} · km^{-1} dépensés par les poissons à migration hâtive) avec, comme résultat, un épuisement plus prononcé des réserves corporelles totales. Cet épuisement plus prononcé peut contribuer à la mortalité plus élevée signalée chez ce composant de la population. Le développement des gonades est terminé avant l'entrée dans la rivière. Il peut ainsi y avoir économie de réserves somatiques lors du jeûne en eau douce et, partant, survie maximale. Nous analysons les caractéristiques de l'utilisation énergétique chez d'autres espèces de poissons anadromes, en

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[1] Contribution of the Huntsman Marine Laboratory.

[2] Present address: North American Salmon Research Center, Huntsman Marine Laboratory, St. Andrews, N.B. E0G 2X0.

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fonction d'une influence possible sur les paramètres démographiques, en particulier la ponte répétée.

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In anadromous fishes the depletion of stored energy reserves during the freshwater migration is commonly extensive as is postspawning mortality (Pacific salmon: Pentegov et al. 1928; Idler and Clemens 1959; Atlantic salmon: Greene 1926; Belding 1934; steelhead: Robertson et al. 1961; Withler 1966; American shad: Glebe and Leggett 1981).

Kalturin (1967) and Schaffer and Elson (1975) have hypothesized that interpopulation differences in the energy content of the body of anadromous fishes at the time of river entry are adaptive and act to compensate for differences in the energy demands of the migration in rivers of different "harshness." Migration distance has also been shown to limit the proportion of repeat spawning shad within and between rivers (Shoubridge and Leggett 1978). These findings suggest that the magnitude of postspawning mortality among anadromous stocks may be strongly linked to the energetic demands of the migration.

Annual intra-population differences in postreproductive survival may also be regulated, in some species, by energy constraints. Leggett (1969) found that Connecticut River shad migrating late in the spawning run when water temperatures are highest suffer higher mortality; and Shoubridge and Leggett (1978) reported that 56% of the annual variability in postspawning survival of shad in the Connecticut River during the interval 1965–72 was due to differences in average river water temperatures during the migration. Since temperatures were never directly lethal, they hypothesized that greater energy use at higher temperatures was an important regulator of survival.

The extent and dynamics of energy use in anadromous fish and their importance as determinants of the demographic characteristics of populations are poorly understood. Only the energetics of the migrations of Fraser River sockeye have been studied in detail (Idler and Clemens 1959). Hypotheses related to energy use thus require further empirical evaluation.

In this paper we report the results of a study of the energetics of the freshwater migration of the American shad (*Alosa sapidissima*) population of the Connecticut River, CT. Our objectives were to examine the sources of energy for the migration, the extent of their utilization, and the effects of differences in water temperatures (as regulated by the timing of river entry) on energy use and survival during the migration.

Materials and Methods

SAMPLING REGIME

Male shad typically precede females into rivers to spawn. We therefore made discrete collections of male and female shad at the peak of the spawning migration and early and late in the run. This temporal separation was achieved by comparing trends in the daily catch per unit effort (C/UE) of males and females in experimental drift gill nets fished at the river mouth from 1967 to 1971 with the daily C/UE of the same gear fished during our study (1972–73). We were thus able to predict the approaching peak and termination of the entry of male and female shad at the river mouth and we timed our sampling to these events. Sampling of early migrants was initiated immediately after their first appearance in the experimental gillnet catches. We subsequently sampled the same segment of the population (early, peak, late migrants) at upriver stations by adjusting the timing of our sampling there to account for known freshwater migration rates (Leggett 1976).

We analyzed 715 shad collected from five Connecticut River stations (Fig. 1) located along the migration route from the river mouth to the Holyoke Dam, the approximate upper limit of the migration for most of the population (Marcy 1972). Unspawned shad were collected at Saybrook (km 0), Rocky Hill (km 50), Wilson (km 83) and Holyoke (km 137). Spent downriver migrants were collected at Brockway Island (km 265), the approximate upper limit of saltwater intrusion into the estuary. The major spawning areas in the Connecticut River are located between km 50 and 137 (Marcy 1976). All shad, with the exception of those sampled at Holyoke, were collected with drift gill nets (11.0 and 14.0 cm strech mesh). Holyoke samples were obtained from the fish lift operated by the Holyoke Water Power Company. At each station we selected male and female shad with the restriction that our samples contained fish that spanned the known length ranges for the two sexes in the population (♂ 43–51 cm; ♀ 44–55 cm). Only unspent fish from catches of upriver migrants and fully spent fish from catches of downriver migrants were analyzed.

All fish were sealed in polyethylene bags and returned to the laboratory within 6 h of capture. For longer delays (maximum 12 h) the bags were packed in ice. Fork length was determined to the nearest 0.5 cm; gonad, viscera, and somatic (eviscerated body) weights to the nearest 1 g. Somatic tissue was homogenized in a 4-L Waring Blendor and ~400 g of somatic homogenate, and the entire gonad and viscera were individually sealed in evacuated polyethylene bags and stored at −34°C until chemical analyses were performed. Age and spawning history were determined by scale reading (Cating 1953; Judy 1961).

BIOCHEMICAL ANALYSIS OF TISSUE SAMPLES

Proximate analyses of total protein, fat, and moisture were performed on the gonad, viscera, and somatic tissue of all shad collected in 1972. These analyses showed that somatic tissues supplied over 90% of the migratory energy requirements and that changes in gonad energy prior to spawning were small (3% of total somatic and viscera energy). We therefore analyzed only the somatic tissues of the samples obtained in 1973. Gonads and viscera were homogenized and somatic tissues rehomogenized prior to analysis. Moisture was determined by drying 5- to 20-g samples to a constant

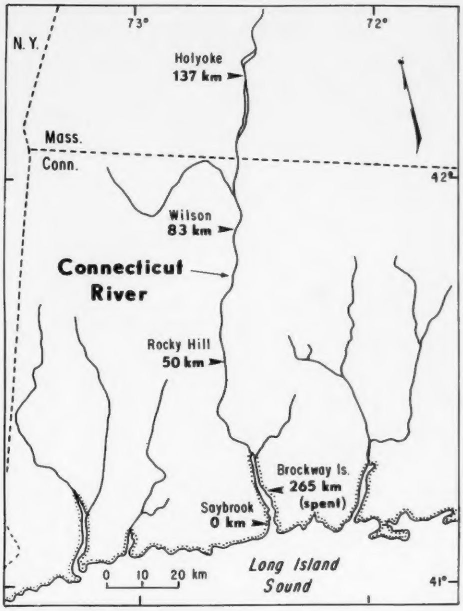

FIG. 1. Location of sampling sites and approximate migration distances to these sites on the Connecticut River.

weight at 90°C. Fat content of the dried viscera and somatic tissue was quantified by the method of Korn and Macedo (1973). Gonad fat was extracted with diethyl ether in a Soxhlet apparatus. Total nitrogen was determined by the nesslerization of Kjeldahl digests (Middleton 1960) of 1.5-g wet samples using selenium as a catalyst (Strickland and Parsons 1972). Protein content was estimated by multiplying total nitrogen values by 6.25 (Dowgiallo 1975).

Energy expenditures and reallocations were determined by applying caloric equivalents (2.4×10^4 J/g for protein; 4.0×10^4 J/g for fat) (Kleiber 1975) to changes in the energy yielding constituents of the gonad, viscera, and somatic tissues.

TISSUE WEIGHT AND ENERGY DYNAMICS

Weight and energy changes in the gonad, viscera, and somatic tissues during the migration were quantified by first deriving linear regressions (logarithmic transformations and polynominal regressions yielded no reduction in residual variance) for shad collected at each location along the migration route. The statistical significance of differences in weight and energy content of the tissues at subsequent stages in the migration was determined by comparing slopes and adjusted means of the appropriate regressions using analysis of covariance (Snedecor and Cochran 1967). Because the variability in energy content at any length was greater than variability in tissue weight (resulting from compounding effect of summing protein and fat energy components), fewer length/tissue energy content regressions than length/tissue weight regressions were statistically significant. Analysis of covariance remained valid however, even for comparison of nonsignificant linear regressions. Only the robustness of the comparison was reduced and some significant differences in slope or elevation may not have been detected as a result. Absolute tissue energy changes were determined for "standard" fish corresponding in length to the calculated mean length of males (45 cm) and females (50 cm) in the population.

The extent of gonad development at the time of entry into freshwater was determined by expressing gonad weight as a proportion of somatic weight (Vladykov 1956) for shad collected at the river mouth.

Results

TISSUE WEIGHT DYNAMICS

A. Gonad weight changes — Only females showed a significant temporal variation in the gonadosomatic ratio at the time of river entry ($P < 0.05$). The mean gonadosomatic ratio of females entering the river early (0.14, SD = 0.03) and late (0.13, SD = 0.02) in the run in 1973 was less than that of shad entering the river at the peak (0.16, SD = 0.03) in 1972.

Subsequent gonad weight changes during the upriver migration occurred only among females. Early and late run females migrating in 1973 exhibited significant increases ($P < 0.01$) in ovarian weights averaging 30% during the upriver migration. The greatest increases were associated with females having the lowest gonadosomatic ratio at river entry. No significant increases in ovarian weight occurred among females migrating at the peak of the run in 1972 ($P > 0.05$).

Spawning of individual shad appeared to be protracted and to occur over a large area. The first gonad weight losses (indicative of spawning) among early migrants in 1973 and peak migrants in 1972 occurred 83 km or more from the river mouth (Fig. 2) and continued to km 137. Late migrant males and females (1973) initiated their spawning closer to the river mouth; the first significant reductions in gonad weight occurring between km 50 and 83.

B. Viscera weight changes — Viscera weight losses were substantially lower in early migrating females (~35% loss to the spawning grounds) than in females migrating at the peak and late in the spawning run (40–60% loss). No significant reduction in viscera weight occurred in any of the three groups following spawning (Fig. 3).

The magnitudes of male viscera weight changes were similar to those changes occurring among females both before and after spawning.

C. Somatic weight changes — Somatic weight losses during the migration from the river mouth to the spawning grounds and return ranged from 30 to 40%. Males and females did not differ significantly in the pattern or magnitude of somatic weight loss (Fig. 4). The three groups (early, peak, and late migrants) differed significantly in the amount of weight lost as measured by the elevation of length/somatic weight regressions. Both male and female shad migrating at the peak of the run had significantly greater somatic reserves

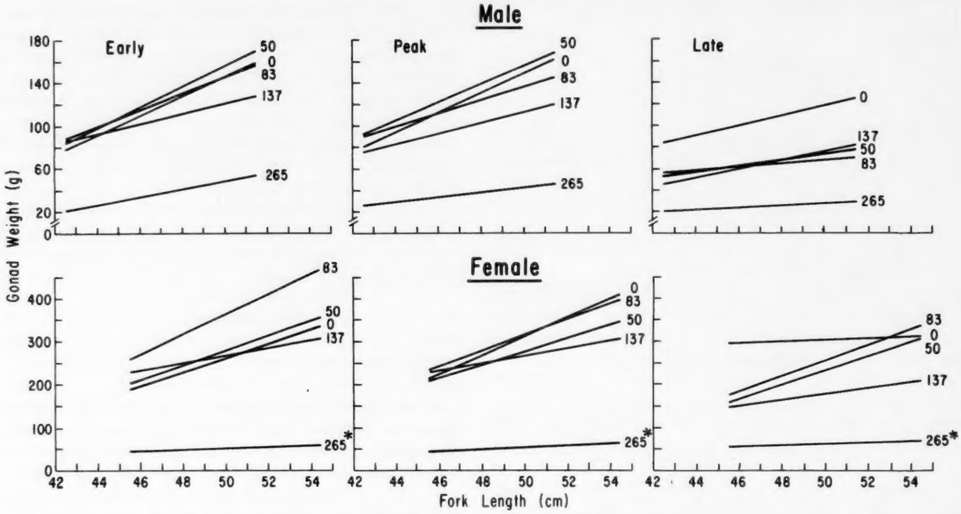

FIG. 2. Gonad weight/body length relationships along the freshwater migration route. (All regressions significant at $P < 0.05$ level except as indicated by * where $0.05 < P < 0.10$. This convention repeated in all subsequent figures.)

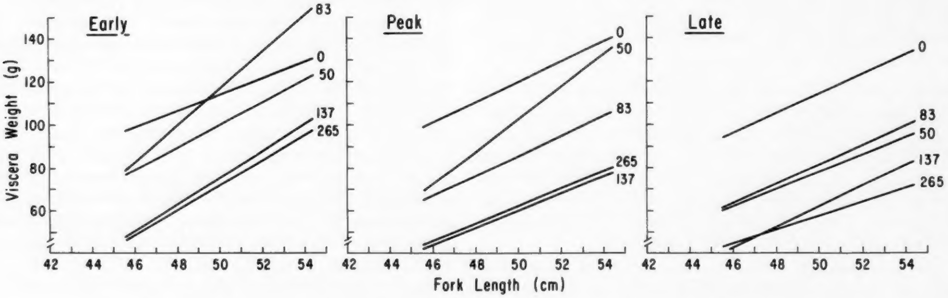

FIG. 3. Female viscera weight/body length relationships at sites along the freshwater migration.

than did shad migrating early ($P < 0.01$) or late ($P < 0.01$). At the spawning grounds the significant differences between the length/somatic weight regressions for early and peak migrants no longer prevailed ($P > 0.05$) while the elevation of this relationship for late migrating shad was significantly below that of both the early ($P < 0.01$) and peak ($P < 0.01$) migrants. This pattern prevailed among spent shad from the three groups when they were subsequently sampled near the river mouth.

D. Interactive changes in tissue weight — Sequential tissue utilization occurred during the prespawning and postspawning migration. In shad migrating early and at the peak of the spawning run, significant reductions in somatic tissue weights occurred only after viscera weight losses reached their maximum. Late migrating shad, in contrast, experienced simultaneous reductions in viscera and somatic weights (Fig. 3, 4). Viscera weight losses occurred principally during the first 50−80 km of the upstream migration (Fig. 3). During the postspawning downstream migration only the somatic tissues experienced a net change in weight (Fig. 3, 4).

PROXIMATE COMPOSITION

A. Fat — Ovarian fat content was relatively constant (5−6 g and 1−2 g for the standard male and female, respectively) during the upriver migration. A sharp decline in fat content (females 85%; males 70%) occurred at spawning (Fig. 5).

Viscera fat content of upstream migrants sampled at the river mouth exhibited significant sex-related differences. Males, despite their smaller size, had three times the absolute viscera fat reserves of females. Viscera fat reserves declined rapidly during the upriver migration, with both sexes con-

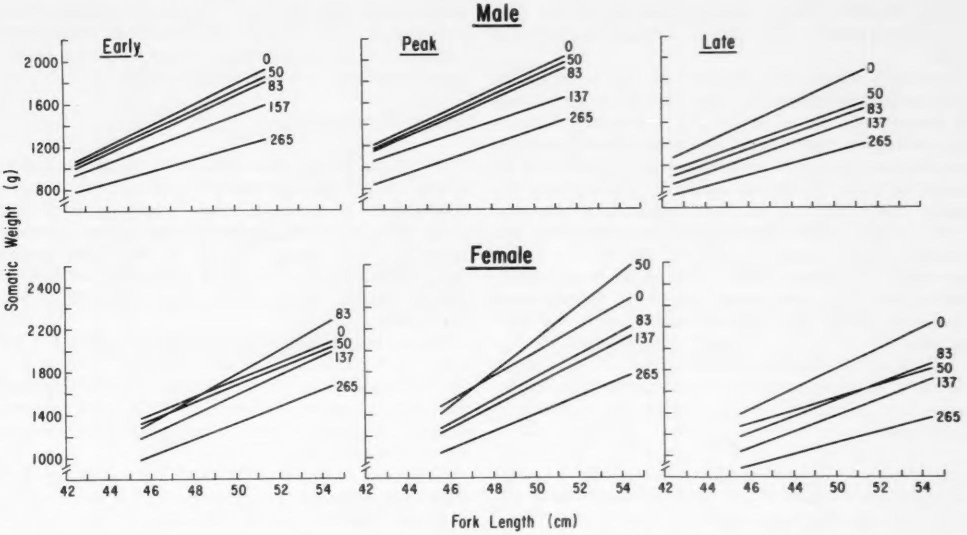

FIG. 4. Somatic weight/body length relationships at sites along the freshwater migration.

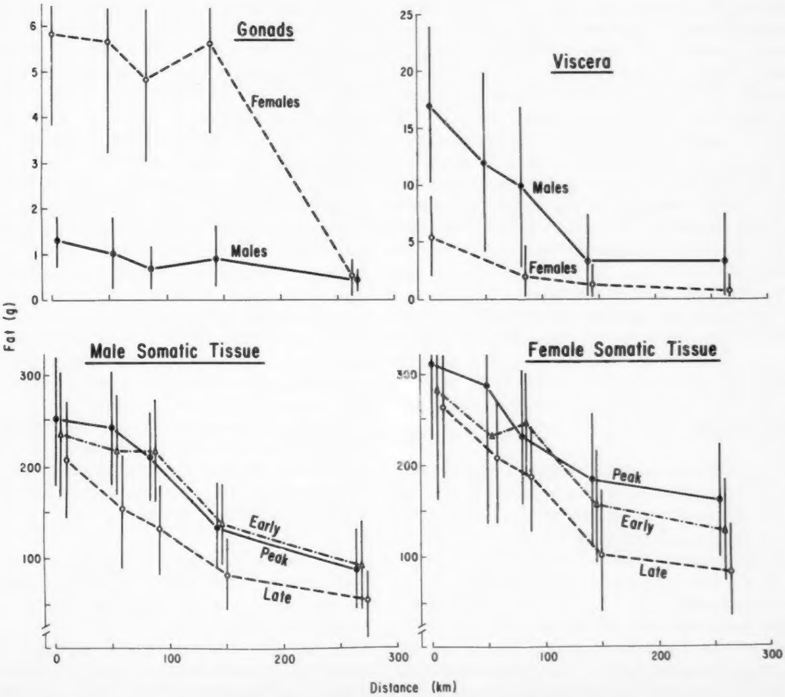

FIG. 5. Tissue fat content of standard shad at various stages of freshwater migration.

suming 70–80% of these reserves during this period. Subsequent to spawning, little change occurred in viscera fat levels.

In contrast, somatic fat content at river entry was greater in females than in males (Fig. 5). This, of course, simply reflects the greater body size of females. On a percentage basis, a rapid, and approximately linear, decline in somatic fat content occurred throughout the upriver migration. Reductions in fat content were less pronounced during the postspawning migration. Early and peak run males and females consumed ~40% of their total somatic fat reserves during the prespawning migration and an additional 10–30% thereafter. Late migrants suffered a higher (60%) reduction in somatic reserves during the prespawning migration. Postspawning losses were similar (10–30%) to those of early and peak migrants.

Somatic tissues were the principal sources of fat during the migration, supplying up to 150 g for metabolic purposes. By comparison, a maximum of 12 g of fat was supplied by viscera reserves.

B. Protein — As with fat, the protein content of ovaries and testes was relatively stable during the prespawning migration (Fig. 6). An abrupt decline (71% for males; 85% for females) occurred at spawning.

Viscera protein content declined by ~45% in both sexes during the prespawning migration (Fig. 6). No change in viscera protein content occurred after spawning. This pattern, too, paralleled the changes in viscera fat reserves. The absolute reduction in viscera protein reserves during the upstream migration averaged 6 g for males and 10 g for females.

Somatic protein declined both before and after spawning, with similar reductions during both intervals (Fig. 6). The decrease in protein content of early migrating females (14%) was comparable to losses suffered by "peak" migrant females (17%). Late migrating females entered the river with a protein reserve similar to that of early migrants but subsequent losses during the migration were greater (30%). The same temporal trends in protein loss existed for males. The average protein content of "early" and "peak" migrant males decreased 18% during the upriver migration between 0 and 137 km and 6% during the downstream migration between 137 km and 265 km. Late male migrants entered the river with reduced protein reserves. Reduction in protein reserves in this group (23 and 14% between 0 and 137 km and between 137 km and 265 km, respectively) were more extensive than those of earlier migrants.

C. Moisture — Ovarian moisture content increased from 71% at river entry to 78% immediately prior to spawning (Fig. 7). Testicular moisture content remained constant at ~78% throughout the migration (Fig. 7).

Viscera moisture content increased ~8% in males and 3% in females during the migration. The smaller increase in females resulted primarily from a higher initial water content in their viscera tissues (75% in females vs. 64% in males).

Somatic tissue water content increased throughout the migration (average increase 7–8%). Late migrant male and female shad had higher average somatic moisture contents and experienced greater increases in moisture content than did early or peak migrants (Fig. 7). In general, changes in moisture content were inversely proportional to changes in fat content. This pattern among fat-depleted fish has been observed previously (reviewed in Love 1970).

ENERGY DYNAMICS

A. Tissue energy relationships — Testicular and ovarian energy content remained relatively stable during the upriver migration to the spawning areas. An abrupt (♂ 60–80%; ♀ 80–85%) reduction in gonad energy content occurred at spawning. The energy content of the testes averaged 5.0×10^5 J (SD = 1.5×10^5) at river entry; the equivalent energy content of the ovary was 1.6×10^6 J (SD = 5.2×10^5).

Viscera energy content at river entry averaged 9.5×10^5 J (SD = 3.5×10^5) in males and 5.9×10^5 J (SD = 1.8×10^5) in females. Use of viscera energy occurred only during the upriver migration and averaged 70% in males and 60% in females; no change in viscera energy occurred during the return to sea (Fig. 8).

Somatic tissues were the principal source of energy for the freshwater migration, providing over 90% of the total energy expended (Fig. 9). Temporal differences in the size of the somatic energy reserve and the proportion of this reserve used during the migration were evident. Male and female shad entering the river at the peak of the spawning migration in 1972 had the greatest reserves averaging 1.6×10^7 J (SD = 4.2×10^6) and 2.1×10^7 J (SD = 4.4×10^6), respectively, for fish of standard length. These reserves were significantly larger than were the reserves of early migrants in 1973 (♂ 1.5×10^7 J, $P < 0.01$; ♀ 1.8×10^7 J, $P < 0.05$) and of late migrants (♂ 1.0×10^7 J, $P < 0.01$; ♀ 1.7×10^7 J, $P < 0.05$) of equivalent length which did not differ significantly one from another ($P > 0.05$).

Male shad migrating early and at the peak of the run expended an average of 50% of their somatic energy reserves during the migration; 30% to reach the spawning area and an additional 20% of return to salt water. Females migrating during the same intervals used a lesser proportion of their somatic reserves averaging 26% to reach the spawning grounds and an additional 20% to return to salt water for a total expenditure of 45% of somatic reserves. Late migrating male and female shad experienced higher energy demands and consumed 60% of their total somatic energy reserves during the complete freshwater migration.

In general, smaller shad consumed a greater proportion of their somatic and total energy reserves to complete the migration than did larger shad. This was true both within sexes and when the males, which generally mature at a smaller size, were contrasted with females.

B. Absolute energy requirements of migration — The upriver energy expenditures of shad migrating in the Connecticut River, calculated by summing viscera and somatic energy use, ranged from 1.9×10^4 to 3.8×10^4 $\mathrm{J/kg \cdot km^{-1}}$ (Fig. 10). The energetic cost of migration increased as the spawning migration progressed and as higher water temperatures were encountered. Late migrating male and female shad experienced energy costs 42 and 87% higher, re-

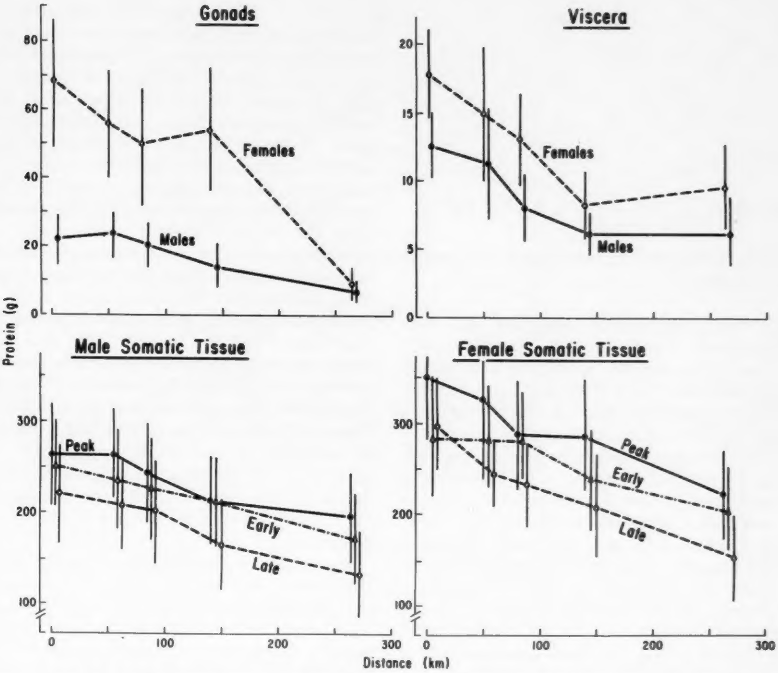

FIG. 6. Tissue protein content of standard shad at various stages of the freshwater migration.

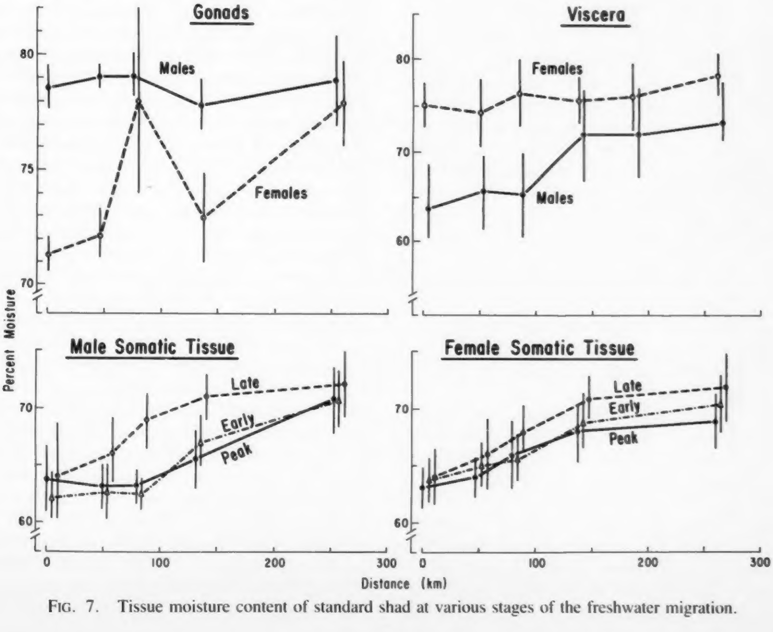

FIG. 7. Tissue moisture content of standard shad at various stages of the freshwater migration.

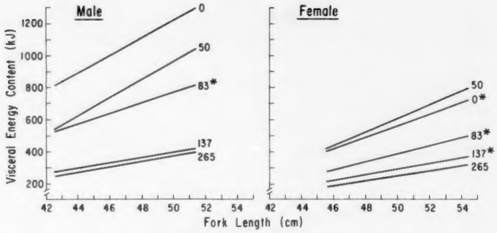

FIG. 8. Viscera energy/body length relationships at sites along the freshwater migration route.

spectively, than early migrants of equivalent sex. Peak migrant male and female shad experienced costs only slightly greater than those of the early migrating group. The energy cost of upriver migration was consistently higher in male shad. The energy expenditures associated with downriver movement could not be calculated since the duration of stay at the spawning areas is not known and energy reserves utilized at this time of meandering would be confounded with energy consumed during directed downriver movement. The sampling of spent shad at departure from the river did, however, provide an estimate of the mean expenditure of energy (viscera and somatic sources combined) from entry into freshwater to this time. The proportion of total energy utilized reflected the seasonal variation in costs associated with upriver movement and ranged from 43 to 62% of the initial energy content of the body (Fig. 10); males generally suffered more extensive energy use.

Discussion

The tissue weight losses and associated energy expenditures during the freshwater migration are both extensive and variable. The absence of gonad development in freshwater moderates the energy requirement during the freshwater period. Shad migrating late in the run and at significantly higher water temperatures expend energy at a greater rate, resulting in more extensive overall depletion. Higher mortalities among late migrants, as suggested by reduced tag returns from these individuals (Leggett 1972), indicates depletion may be a factor limiting postreproductive survival and determinant of the demographic characteristics of shad populations in general. This close relationship between water temperature during migration, energy use, and mortality also suggests that energy allocation to migration vs. reproduction is delicately adjusted with the maximum possible allocation being made to reproduction.

The variation in the female gonadosomatic ratio at river entry suggests environmental factors may be differentially influencing the timing of ovarian development between years. Shad from all Atlantic Coast stocks migrate together at sea (Talbot and Sykes 1958; Leggett 1977; M. Dadswell, Fisheries and Oceans Canada, St. Andrews, N.B., personal communication). Leggett and Whitney (1972) found that while at sea, the geographical location of these stocks is determined by their preference for specific water temperatures. Annual variation in the latitudinal position of waters of preferred temperatures on specific dates could thus influence the photoperiod experienced. Since both temperature and photoperiod are known to be important in the neuroendocrine mediation of maturation in fish (De Vlaming 1978), annual variation in the state of ovarian development at arrival in the home river would be expected. Tissue component analysis which indicated subsequent increases in ovarian weight during the upriver migration in 1973 comprised only an increase in moisture content. No relocation of stored energy to the gonads was observed. This pattern of energy allocation differs markedly from that reported for Pacific and Atlantic salmon which typically allocate significant amounts of stored body reserves for gonad maturation during the freshwater migration (Greene 1926; Belding 1934; Idler and Clemens 1959) and from shad populations spawning in the southern portion of the Atlantic coast range (Glebe and Leggett 1981). This pronounced difference between the transfer of somatic and viscera energy reserves to the gonads of Connecticut River shad, southern shad populations, and salmon is paralleled by equally pronounced differences in the frequency of repeat reproduction in the two groups (Connecticut River shad 30%, Leggett 1976; St. Johns River, Fla. shad 0%, Leggett and Carscadden 1978; Atlantic salmon 7% (average of 14 rivers), Schaffer and Elson 1975; pacific salmon 0%) and may account, in part, for these differences.

The pattern of gonad weight and energy losses associated with upriver movement is consistent with reported intermittent spawning (Camfield 1937, cited in Walburg and Nichols 1967). Individual shad apparently spawn repeatedly as they progress upriver thereby producing a gradual reduction both in weight and energy content of the testes and ovaries. This spawning mode may be necessary to facilitate the high fecundity of shad. Both intermittent and complete spawning species are found in the family Clupeidae (Blaxter and Holliday 1963). Naumov (1956) found that the total fecundity of the intermittent spawning Caspialosa species was higher than in the Murmansk herring which spawn totally on one occasion. Shoubridge and Leggett (1978) have also shown that in American shad a two- to four-fold increase in body cavity volume would be required to accommodate an equivalent number of eggs if all eggs reached full volume and were spawned at one time. The finding that, in this population, little or no energy is transferred to the gonad while the shad are in freshwater clearly indicates, however, that the increase in egg volume which occurs just before spawning is due to the incorporation of water and not of fat and protein at this time.

Gonad weight and energy reductions closer to the river mouth by late run migrants in 1973 suggests the higher water temperatures encountered may be initiating spawning earlier in the upriver migration. Walburg and Nichols (1967) and R. E. Painter (California Department of Fish and Game, 1977, unpublished manuscript) reported peaks of spawning activity coincided with temperature modes of from 14 to 21°C and Marcy (1976) was unable to find eggs in abundance below 12°C. Early migrant shad do not encounter water temperatures of this magnitutde until Holyoke is reached. The first indication of spawning (gonad weight loss) does not occur until this time. Late migrants, however, experience water temperatures averaging 18°C in the vicinity of Rocky Hill, 87 km downstream from Holyoke. Spawning is initiated at this point and continues intermittently as upriver progress is made.

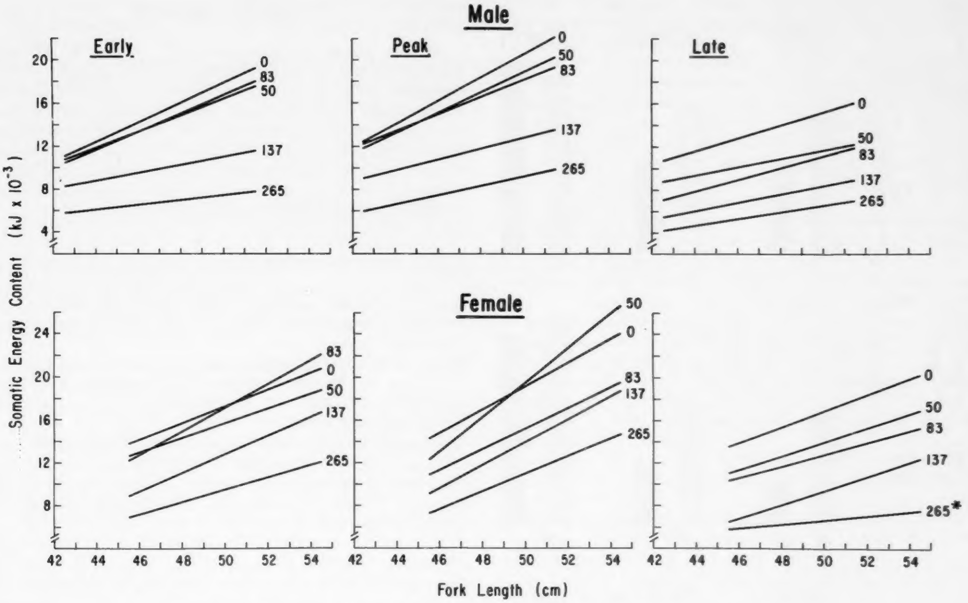

FIG. 9. Somatic energy/body length relationships at sites along the freshwater migration route.

In shad, the total energy content of the testes is less than that of the ovaries (5.4×10^5 J vs. 1.6×10^6 J for the standard length male (45 cm) and female (50 cm) gonad, respectively) and the relative cost of gonad development is reflected in the energy stores of the two sexes. Males require much less energy for the maturation of the gonads. This could explain the higher somatic and viscera energy reserves of males relative to females at the time of entry into freshwater. Sex-based differences in the allocation of energy to gonad development have been reported for salmon (Belding 1934; Idler and Bitners 1958), cod (Kordyl 1951), herring (Blaxter and Holliday 1963), and lampreys. However, Ursin (1979) has cautioned against a ready acceptance of this argument. The cost of gonad maturation is not necessarily accurately reflected in the absolute energy containment of the gonad. Testicular tissue consists almost entirely of DNA, RNA, and lipids and relative to ovarian tissue may be metabolically more costly to produce.

Subsequent proportional energy expenditures associated with upriver movement are greatest among males. Depending on the timing of migration, energy requirements by males are 15–35% greater than that of females. The generally smaller size of males presumably reduces metabolic efficiency in terms of energy expended per unit body weight during active swimming (Winberg 1956). The discrepancy in energy usage by males and females may reflect size-related energy efficiencies. In contrast, female Fraser River sockeye salmon expend 17% more energy than males during the migration (Idler and Bitners 1965). The greater cost to females is related more to extensive ovarian development in freshwater rather than size-related swimming efficiency.

Differences in migration behavior are evident when the rate of energy expenditure for shad and Pacific salmon are compared. A cost of $20-37\ \mathrm{kJ \cdot kg^{-1} \cdot km^{-1}}$ reflects the slow (6 km/d) meandering upriver movement by shad (Leggett 1976; Leggett and Trump 1978). While a more direct migration by two Pacific salmon species (33 km/d, Idler and Clemens 1959; 43 km/d, Pentegov et al. 1928) results in reduced energy usage per unit of upriver displacement (4.6 and $3.5\ \mathrm{kJ \cdot kg^{-1} \cdot km^{-1}}$ for female and male sockeye, respectively; 2.7 and $2.9\ \mathrm{kJ \cdot kg^{-1} \cdot km^{-1}}$ for male and female chum, respectively). The lower rate for Amur River chum may be due to size-mediated metabolic expenditures. Their average size of 5 kg is significantly greater than that of the Fraser River sockeye (3 kg) and Connecticut River shad (2 kg). Similarly Schaffer and Elson (1975) have implied that large Atlantic salmon may have a metabolic advantage when the freshwater migration is particularly "harsh" or long.

Leggett (1972) and Chittenden (1969) have suggested that the extensive expenditure during the freshwater migration of American shad may be a factor influencing certain aspects of their demography, especially the latitudinal variation in the proportion of repeat spawners. The more extensive energy depletion by late run Connecticut River shad (≈ 60% of initial total reserves) is clearly correlated with the higher spawning mortality within this segment of the population (Leggett 1969). Year-to-year variations in the proportion of repeat spawners in the Connecticut River population have also been shown to be strongly negatively correlated with average water temperatures during the migration 1 yr earlier (Shoubridge and Leggett 1978) again suggesting an energetic effect on demography. The low frequency of repeat reproduction in

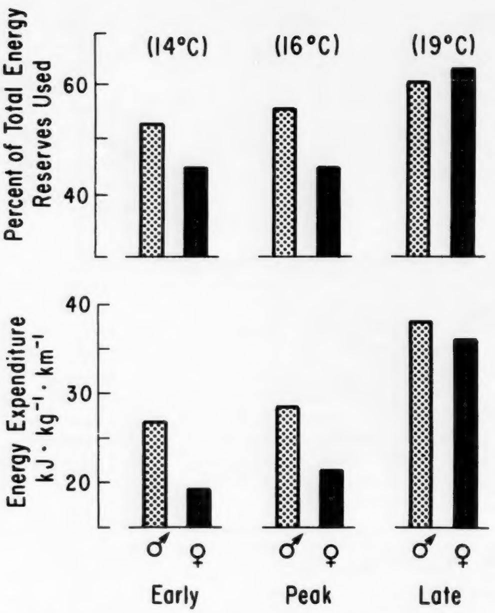

FIG. 10. Absolute and proportional energy usage during the freshwater migration and associated average water temperatures.

shad native to more southern rivers may also be related to higher energy costs of migration (Leggett and Carscadden 1978). However, as Mann and Mills (1979) have noted, the effects of proximate and ultimate factors are often difficult to distinguish in demographic studies. Many hypotheses explaining the differences between populations are plausible but difficult to prove empirically. For example, the utilization of 64–79% of body reserves by Fraser River sockeye and Amur River chum salmon (levels associated with death in other species, Belding 1934) may be construed as being responsible for semelparity in these stocks. However, complete spawning mortality occurs in other stocks (and among pink salmon) where the freshwater migration distance is short and the energy requirement minimal. Ultimate factors such as age-specific mortality and reproductive uncertainty (reviewed by Stearns 1976) may serve a more important role in determing both the pattern of energy expenditure and life history tactics in Pacific salmon and perhaps also in shad. These questions are considered in greater detail in Glebe and Leggett (1981) and Shoubridge and Leggett (1978). The difficulty of distinguishing between proximate and ultimate factors notwithstanding, the patterns of energy allocation between gonad and somatic tissue, and the fine tuning of reproductive vs. somatic energy partitioning as it relates to survival offer strong support for the energy allocation hypothesis advanced by Cody (1966), Gadgil and Bossert (1970), Charnov and Krebs (1973) and others as a functional basis for optimization of life history parameters.

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Latitudinal Differences in Energy Allocation and Use During the Freshwater Migrations of American Shad (*Alosa sapidissima*) and Their Life History Consequences[1]

B. D. GLEBE[2] AND W. C. LEGGETT

Department of Biology, McGill University, Montreal, Que. H3A 1B1

GLEBE, B. D., AND W. C. LEGGETT. 1981. Latitudinal differences in energy allocation and use during the freshwater migrations of American shad (*Alosa sapidissima*) and their life history consequences. Can. J. Fish. Aquat. Sci. 38: 806–820.

We studied the relationships between tissue dynamics and bioenergetics of the anadromous American shad (*Alosa sapidissima*) homing to the St. Johns (Fla.), York (Va.), and Connecticut (Conn.) Rivers and the life history characteristics of these populations. Shad in the three populations studied differed in the degree of development of the gonads at river entry, in the absolute and relative energy allocation to reproductive products vs. migration, and in the extent of total energy depletion during the migration. St. Johns River fish consumed 70–80% of their total energy reserves to reach the spawning grounds and spawn. In this population all shad die following spawning. York River shad consumed ~30% and Connecticut River shad 40–60% of their energy reserves to migrate to the spawning grounds, spawn, and return to the sea. In these populations 25 and 35%, respectively, of the spawning adults survive to spawn again. The principal determinants of energy use were migration distance and river gradient. We rejected the hypothesis that the latitudinal cline in adult survival results from differences in energy use during migration. We concluded that interpopulation differences in energy allocation to migration vs. reproduction are a consequence, rather than a cause, of the different life history strategies exhibited by populations of shad over its Atlantic Coast range. A similar pattern is apparent in other anadromous species: obligate semelparous species use >70% of their energy reserves to reach the spawning areas and spawn; iteroparous species allocate more energy to postreproductive reserves at the expense of reproductive products thereby ensuring a successful return migration to the sea. Freshwater swimming speeds also appear to differ in a consistent way between semelparous and iteroparous species. Semelparous species swim at close to maximum sustained speed thereby minimizing the duration of the migration. Iteroparous species swim at speeds yielding near optimum energy efficiency ($J \cdot kg^{-1} \cdot km^{-1}$) thereby minimizing the energy cost of migraiton.

Key words: American shad, (*Alosa sapidissima*); migration, bioenergetics, life history tactics, latitudinal clines, swimming speeds, energy allocation, anadromy

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Nous avons étudié les relations entre la dynamique et l'énergobiose des tissus de l'alose américaine (*Alosa sapidissima*) anadrome retournant dans leurs rivière d'origine, St. Johns (Fla.), York (Va.) et Connecticut (Conn.), ainsi que les caractéristiques du cycle biologique de ces populations. Les membres des trois populations étudiées diffèrent quant au degré de développement des gonades au moment de l'entrée en rivière, à l'attribution d'énergie absolue et relative aux produits sexuels vs. migration, et enfin au degré d'épuisement énergétique total lors de la migration. Les aloses de la rivière St. Johns consomment de 70 à 80 % de leurs réserves énergétiques totales pour atteindre les frayères et frayer. Dans cette population, les aloses meurent après avoir frayé. Les sujets de la rivière York consomment ~30 % et ceux de la rivière Connecticut 40–60 % de leurs réserves énergétiques pour se rendre sur les frayères, frayer et retourner à la mer. Chez ces populations, 25 et 35 %, respectivement, des

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[1] Contribution of the Huntsman Marine Laboratory.

[2] Present address: North American Salmon Research Center, Huntsman Marine Laboratory, St. Andrews, N.B. E0G 2X0.

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adultes reproducteurs survivent et frayent de nouveau. La durée de la migration et le gradient de la rivière sont les principaux facteurs déterminant l'utilisaiton de l'énergie. Nous rejetons l'hypothèse que le cline latitudinal de survie des adultes résulte de différences dans l'utilisation de l'énergie lors de la migration. Nous concluons que les différences entre les populations dans l'attribution de l'énergie à la migration vs. reproduction sont une conséquence plutôt qu'une cause des différentes caractéristiques du cycle biologique des populations d'aloses savoureuses dans leur aire de distribution le long de la côte atlantique. De semblables caractéristiques sont évidentes chez d'autres espèces anadromes : celles qui ne se reproduisent qu'une fois utilisent >70 % de leurs réserves énergétiques pour atteindre les frayères et frayer; celles qui se reproduisent plus d'une fois attribuent plus d'énergie aux réserves postreproductrices au dépens des produits sexuels, assurant ainsi le succès d'une migration de retour à la mer. Les vitesses de nage semblent également différer de manière uniforme entre les deux types. Les espèces à ponte unique nagent à une vitesse qui se rapproche de la vitesse soutenue maximale, minimisant ainsi la durée de la migration. Les espèce à ponte répétée nagent à des vitesses donnant un rendement énergétique proche de l'optimum ($J \cdot kg^{-1} \cdot km^{-1}$), minimisant ainsi le coût énergétique de la migration.

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NUMEROUS anadromous fish species undertake long freshwater migrations without feeding. The energy required to support these migrations is derived from somatic and viscera reserves accumulated at sea. Extensive weight loss and energy depletion is common and high spawning and postspawning mortality frequently occurs (Pacific salmon: Pentegov et al. 1928; Idler and Clemens 1959; Atlantic salmon: Greene 1926; Belding 1934; steelhead: Robertson et al. 1961; Withler 1966; lamprey: McCay 1931; Beamish 1980; American shad: Chittenden 1969; Leggett 1972).

Khalturin (1967) and Schaffer and Elson (1975) have provided evidence in support of the hypotheses that, in Atlantic salmon (*Salmo salar*), interpopulation differences in the energy content of the body at maturity, inferred from size at maturity data, are adaptive and are related to the "harshness" of the migration. Leggett and Carscadden (1978) and Shoubridge and Leggett (1978) have also proposed that latitudinal differences in adult postspawning survival and fecundity in both Atlantic and Pacific coast populations of the anadromous American shad (*Alosa sapidissima*) may involve differences in energy allocation to reproduction and adult survival. These observations bear directly on the more general relationship of energy allocation patterns to the evolution of life history tactics in plants and animals (reviewed in Stearns 1976).

We believe studies of the energetics of migration in American shad (*A. sapidissima*) can contribute meaningfully to a broader understanding of the importance of energetics in the survival and reproductive ecology of anadromous fishes in particular, and of animals in general. The shad is anadromous, spawns in most major Atlantic coast rivers between St. Johns River, Florida, and the St. Lawrence River, Canada, and powers its freshwater migration with energy reserves accumulated at sea (Leim 1924; Atkinson 1951; Bigelow and Schroeder 1953). Shad home to their natal rivers to spawn (Carscadden and Leggett 1975a, b) and the populations appear to be genetically isolated. The timing of the spawning migration is strongly temperature regulated and hence members of all populations experience similar, although not identical, temperature conditions during the migration (Leggett and Whitney 1972).

Of particular significance to the question of the role of migration energetics in reproduction and survival in anadromous fish is the fact that shad exhibit a pronounced latitudinal cline in postspawning survival; southern populations are semelparous, northern populations are strongly iteroparous (Leggett and Carscadden 1978). Chittenden (1969) and Leggett (1972) have hypothesized that this cline in survival has an energetic basis. In support of this view, Glebe and Leggett (1981) have shown that annual intra-population differences in postreproductive survival are regulated, in part, by energy constraints.

In this paper we present the results of a comparative study of the energetics of the freshwater migration in members of American shad populations spawning at three latitudes (Fig. 1). Our objective, in conducting this work was to examine the relationship between the dynamics of weight loss and energy expenditure in these populations and the pronounced differences in postspawning survival and life history traits (Leggett and Carscadden 1978; Shoubridge and Leggett 1978) that they exhibit; and to use these findings to assess the possibility of an energetic basis for differences in life history patterns in other anadromous species.

Materials and Methods

SAMPLING

We studied the migration energetics of shad populations spawning in the St. Johns (Fla.), York (Va.), and Connecticut (Conn.) Rivers. The life history traits exhibited by these populations reflect the range of traits occurring in Atlantic coast populations (Leggett and Carscadden 1978). All shad used in our analyses were collected near the peak of the spawning run in their respective rivers.

In the Connecticut River we were able to sample the same segment of the run throughout the migration by adjusting our sampling schedule to account for known freshwater migration rates there (Leggett 1976). We analyzed 348 shad collected at five Connecticut River stations (Fig. 1). Unspawned shad

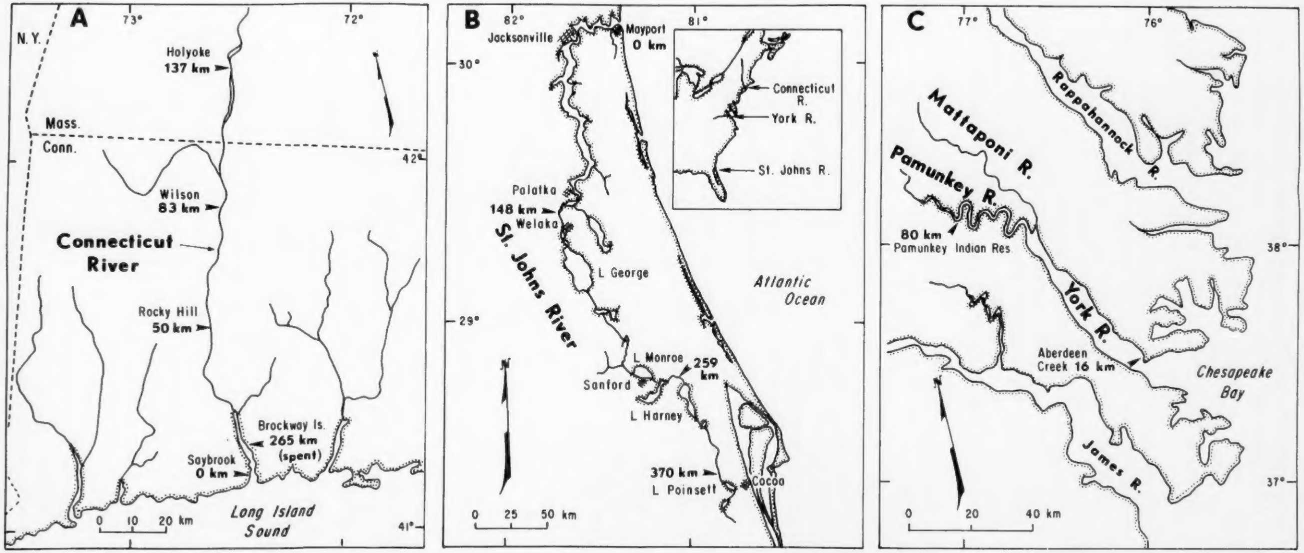
A
73°
72°
N.Y.
Mass.
Conn.
42°
41°
Holyoke
137 km
Wilson
83 km
Connecticut River
Rocky Hill
50 km
Brockway Is.
265 km
(spent)
Saybrook
0 km
Long Island Sound
0 10 20 km
B
82°
81°
30°
29°
Jacksonville
Mayport
0 km
Connecticut R.
York R.
St. Johns R.
Palatka
148 km
Welaka
St. Johns River
L George
Atlantic Ocean
L Monroe
259 km
Sanford
L Harney
370 km
L Poinsett
Cocoa
0 25 50 km
C
77°
76°
38°
37°
Rappahannock R.
Mattaponi R.
Pamunkey R.
80 km
Pamunkey Indian Res
York R.
Aberdeen Creek 16 km
Chesapeake Bay
James R.
0 20 40 km

FIG. 1. Locations of study rivers and sampling sites.

were collected at Saybrook (km 0), Rocky Hill (km 50), Wilson (km 83), and Holyoke (km 137). Spent downriver migrants were sampled at Brockway Island (km 265). All shad, with the exception of those sampled at Holyoke, were collected with 11.0 and 14.0 cm (stretched mesh) drift gill nets. Holyoke samples were taken from the fish lift located at Holyoke Dam.

Migration rates of the St. Johns and York River populations are unknown. We sampled these populations sequentially from the river mouth to the spawning grounds but the extent to which we managed to sample the same segment of the run throughout is unknown. For the St. Johns River population we analyzed 268 shad collected at five stations (Fig. 1) distributed along the migration route between the river mouth and the upper limit of the spawning grounds at Lake Poinsett (Williams and Bruger 1972). Samples of both unspawned and spawned fish were collected at Lake Poinsett. Collection methods, which varied from station to station are summarized below:

Station	Gear
Mayport	11.4, 12.7, and 14.0 cm (stretch mesh) gill nets
Welaka	6.3 cm (stretch mesh) commercial seine
Lake George	11.4 and 12.7 cm (stretch mesh) gill nets
Lake Poinsett	11.4 and 12.7 cm (stretch mesh) gill nets; 5.0 and 25.4 cm (square mesh) trammel net; angling

For the York River population we analyzed 83 shad sampled at two stations, one near the river mouth and the second near the spawning grounds (Fig. 1). All shad were collected with 12.7 cm (stretch mesh) gill nets.

Male and female shad sampled from the St. Johns and Connecticut populations were selected from the respective catches with the sole restriction that the range of lengths for the two sexes in each population were adequately represented (St. Johns ♂ 33−43 cm, ♀ 38−44 cm; Connecticut ♂ 43−51 cm, ♀ 44−55 cm). Only one size of male (36 ± 1.0 cm) and female (42 ± 1.0 cm) shad, representative of the most abundant size-class, was sampled from the York River population. Each sample analyzed from the three populations contained ~20 fish of each sex.

Fish were sealed in polyethylene bags immediately after capture and were processed (see below) and frozen within 6 to 7 h. Where occasional longer processing times were encountered (maximum 12 h) the bags were packed in ice. Prior to freezing fork length was determined to the nearest 0.5 cm; gonad, viscera, and somatic (eviscerated body) weights were recorded to the nearest 1 g, the somatic tissue was homogenized in a 4-L Waring Blendor, and 400 g of the homogenate, the viscera, and the gonads were individually sealed in evacuated polyethylene bags. All tissues were stored at −34°C until proximate analyses were conducted.

PROXIMATE ANALYSES

Determinations of total protein, fat, and moisture content were made for all shad sampled from the St. Johns and Connecticut populations. Gonad and viscera tissues were homogenized and somatic tissue samples were rehomogenized prior to the analyses. Moisture was determined by drying 5- to 20-g samples to a constant weight at 90°C, fat content of the dried viscera and somatic tissues was quantified by the method of Korn and Macedo (1973), and gonad fat was extracted with diethyl ether in a Soxhlet apparatus. Total nitrogen was determined by the nesslerization of Kjeldahl digests (Middleton 1960) of 1.5 g wet samples using selenium as a catalyst (Strickland and Parsons 1972). Intensity of the Nessler color complex was measured on a Unicam SP 600 spectrophotometer using a 4-cm cell. Protein content was estimated by multiplying total nitrogen values by 6.25 (Dowgiallo 1975). All tissue constituent analyses were performed in duplicate.

Energy expenditures and reallocations by Florida and Connecticut shad were determined by applying caloric equivalents (2.4×10^4 J/g for protein; 4.0×10^4 J/g for fat; Kleiber 1975) to changes in the energy-yielding constituents of the gonad, viscera, and somatic tissues. Energy expenditure by York River shad was determined by combustion of 1 g dry samples of gonad, viscera, and somatic tissue in a Parr non-adiabatic oxygen macro-bomb calorimeter. The equivalence of estimates of tissue energy content using the heat of oxidation method and caloric equivalents of tissue fat and protein content was verified by chi-square analyses of data obtained using both methods of 12 tissue samples.

TISSUE WEIGHT AND ENERGY DYNAMICS

Weight and energy changes in gonad, viscera, and somatic tissues during the migration were quantified by deriving linear regressions (logarithmic transformations and polynomial regressions yielded no reduction in residual variance) for shad collected at each sampling station along the migration route. The statistical significance of differences in weight and energy content of the tissues at subsequent stages in the migration was determined by comparing slopes and adjusted means of the appropriate regressions using analysis of covariance (Snedecor and Cochran 1967). Absolute tissue energy changes were determined for fish corresponding in length to the calculated mean length of males and females in the respective populations (Florida ♂ 40 cm, ♀ 42 cm; Virginia ♂ 37 cm, ♀ 42 cm; Connecticut ♂ 45 cm, ♀ 50 cm). Fish of these lengths are referred to as "standard" shad in the text.

The degree of gonad development at river entry was quantified by expressing gonad weight as a proportion of somatic weight (Vladykov 1956).

Results

TISSUE WEIGHT CHANGES

Significant intra- and inter-population differences in the degree of gonad development at the time of river entry were found (Fig. 2). Within populations, the ovaries of large St. Johns River and small Connecticut River shad were less developed than those of other individuals in their respective populations. Differences in the degree of testes development were small. The gonads of St. Johns River shad were significantly less developed than those of Connecticut River

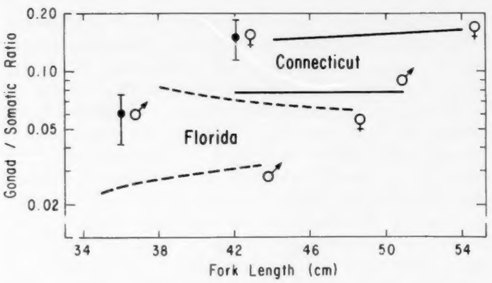

FIG. 2. Ratio of gonad/somatic weight at river entry (St. Johns River, Florida (---); York River, Virginia (●); Connecticut River, Connecticut (—).

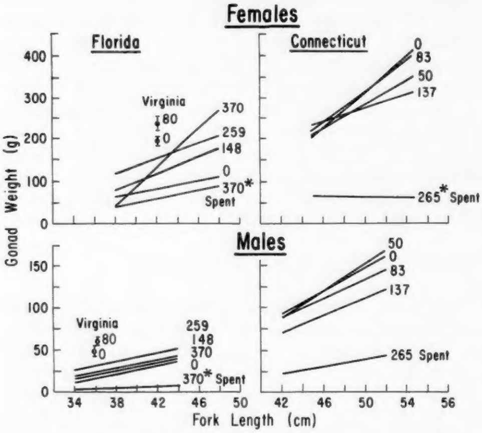

FIG. 3. Gonad weight/body length relationships at various stages of the freshwater migration. (All regressions significant at $P < 0.05$ level except as indicated by * where $0.05 < P < 0.10$. This convention repeated in all subsequent figures.)

migrants at river entry ($P < 0.01$). The stage of maturity of the gonads of York River shad was similar to that of Connecticut River shad (Fig. 2).

St. Johns and York River shad experienced a significant increase in both testicular and ovarian weights during the upriver migration to the spawning areas (Fig. 3). The increase in testicular and ovarian weights in Florida shad ranged from 40 to 100%, depending on the size of the individual. Increase in gonad weight experienced by York River shad (♂ 20%, ♀ 21%) was considerably less than the smallest gonad weight increases occurring in Florida shad. No change in gonad weight occurred in Connecticut River shad during the upriver migration to the spawning areas.

In Florida the continued increase in the gonad weight of both sexes to 259 km indicated that little or no spawning occurred below this point. A subsequent significant decrease ($P < 0.01$) in the length/gonad weight relationship at 370 km suggested some spawning had occurred between km 259 and 370. This finding is consistent with the reported distribution of spawning areas in the St. Johns River (Williams and Bruger 1972). Visual comparison of the testes of these male shad with those of fully spent males sampled later at the same location (370 km) indicated spawning in the former group was incomplete at the time of sampling. The proportional decrease in ovary weights of females less than 43 cm beyond km 259 and the significant increase in slope of the length/ovary weight relationship at km 370 ($P < 0.05$) indicated that the smaller females (which were more mature at time of entry into freshwater) were spawning earlier and further downstream than their larger counterparts.

Spawning of individual Connecticut River shad appeared to be protracted and to occur over a large area. Gonad weight losses indicative of significant spawning first occurred ~50–83 km from the river mouth (Fig. 3).

The significant increase in gonad weight of Virginia shad between the river mouth and km 80 suggested little or no spawning occurred downstream of km 80. This finding agrees with the reported limited distribution of spawning activity in the York River system (Massmann 1952).

Significant interpopulation differences were observed in viscera weights at the time of river entry. The length/viscera weight relationship of Florida shad was significantly below that of Connecticut migrants ($P < 0.01$) indicating they enter freshwater with smaller viscera energy reserves. Viscera weight losses in St. Johns River females during the upriver migration (~35% of initial levels) were substantially less than viscera weight losses in Connecticut River females (40–60%) (Fig. 4). The smaller proportional weight loss of Florida females was apparently a consequence of the reduced viscera reserves carried into the river because the slope ($P > 0.05$) and elevation ($P > 0.05$) of the length/viscera weight relationships of the Connecticut and Florida shad did not differ at the spawning grounds. For males, the viscera weights at river entry in the two populations were more similar and, consequently, proportional viscera weight losses during the upriver migration were also similar (Fig. 4). The lack of significant decreases in viscera weight in both populations after spawning (Fig. 4) suggests that shad exploit their viscera reserves maximally during the upriver migration.

Mean viscrea weights at river entry in the York River population (36 cm ♂: 48 g, SD = 9, n = 21; 42 cm ♀: 67 g, SD = 10, n = 22) were slightly greater than the viscera weights of Florida shad of equivalent lengths. Viscera weight losses during the upriver migration (♂ 35%; ♀ 14%) were substantially less than losses by either Florida or Connecticut shad during travel to their respective major spawning sites. Some viscera reserves may thus be available for the downstream migration of York River shad.

No significant differences occurred in the slope ($P > 0.05$) or elevation ($P > 0.05$) of the length/somatic weight relationship of males and females from the Florida and Connecticut populations at the time of entry into the rivers. Mean somatic weights of males (790 g, SD = 71, n = 21) and females (1202 g, SD = 76, n = 22) entering the York River system approximated the weights of Florida males (758 g) and females (1165 g) of comparable lengths entering the St. Johns River. Somatic weight losses experienced in attaining the spawning grounds were similar in Florida and Connecticut (15–25%; Fig. 5) in spite of the greater distances travelled by Florida fish. In Virginia, where the spawning migration is

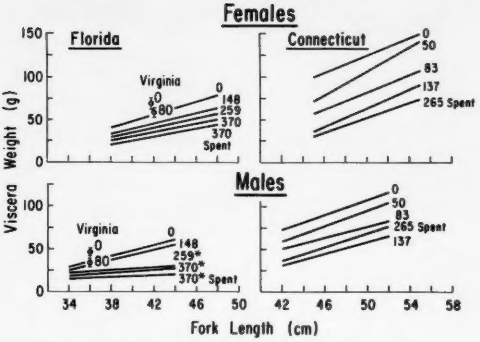

FIG. 4. Viscera weight/body length relationships at various stages of the freshwater migration.

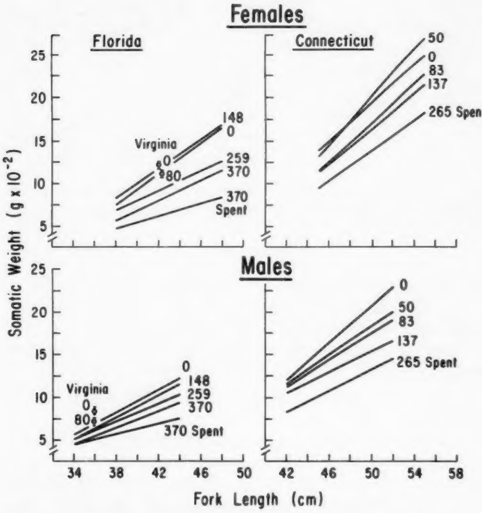

FIG. 5. Somatic weight/body length relationships at various stages of the freshwater migration.

shortest, the magnitude of the somatic weight loss associated with the migration to the spawning area was less (~8%).

During the spawning and postspawning period St. Johns River shad experienced weight losses in excess of those occurring in the Connecticut fish. St. Johns River shad sampled on the spawning grounds had experienced proportional weight losses as great (males) or greater than (females) spent Connecticut River fish sampled at the river mouth just prior to the return to the sea. Florida males and females had consumed 20−35% and 40−50%, respectively, of their somatic tissues during the period from river entry to spawning. Weight losses of Connecticut River shad during the entire freshwater migration including the postspawning return to the sea ranged from 30 to 40% (Fig. 5). No comparable data is available for York River migrants.

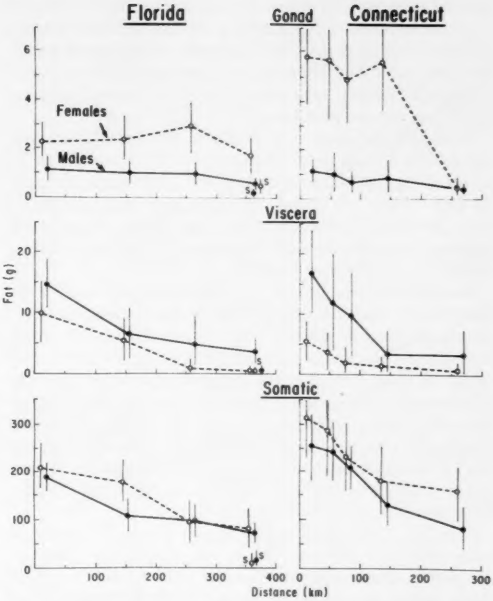

FIG. 6. Tissue fat content of standard shad at various stages of the freshwater migration (S, St. Johns River spent shad).

INTERACTIVE CHANGES IN TISSUE WEIGHT

Connecticut River shad exploited their available tissue reserves differentially. Viscera reserves were used almost exclusively during the first 50 km of the migration (Fig. 4). Significant reductions in somatic weights occurred only after viscera weight loss had reached its maximum level (Fig. 5). Somatic tissue alone was consumed during the downriver migration (Fig. 4, 5).

St. Johns River shad utilized somatic and viscera reserves simultaneously during the upriver migration. Following spawning only somatic tissues were utilized (Fig. 4, 5).

The limited number of sampling locations in the York River prevented a definitive assessment of interactive changes in shad viscera and somatic weights during the upstream migration of that population. The smaller viscera and somatic weight losses experienced by Virginia migrants relative to losses experienced by Connecticut and Florida shad during travel to spawning sites does, however, suggest that, unlike Connecticut and Florida migrants, Virginia shad may have both viscera and somatic energy reserves available for the postspawning migration.

PROXIMATE COMPOSITION

Trends in gonad fat changes during the migration were similar in male and female Connecticut River shad, although the absolute levels of fat in the gonads and the magnitude of the changes in fat levels following spawning differed markedly between sexes (Fig. 6). Ovarian fat content remained quite constant during the upriver migration (5−6 g for "standard" females). At spawning ovarian fat levels dropped sharply to ~15% of prespawning levels. Testicular fat levels, too, remained quite stable prior to spawning (0.8−1.2 g for "standard" males). At spawning testicular fat levels declined to 70% of prespawning levels.

Testicular fat levels were higher in St. Johns River shad, the smaller Florida males (standard length 40 cm) having fat levels (1.1 g) equivalent to those of the larger (standard length 45 cm) Connecticut males. Trends in testicular fat changes were, however, similar in the two populations. Prespawning fat levels were largely stable. At spawning fat levels declined to 68% of prespawning levels.

Ovarian fat levels at river entry were markedly lower in St. Johns River shad, relative to those of the Connecticut River population both in absolute (Fig. 6) and relative (St. Johns 0.17 g/g somatic tissue; Connecticut 0.30 g/g somatic tissue) terms. In sharp contrast to the prespawning ovarian fat levels of Connecticut River shad, however, those of the St. Johns River shad increased by an average of 35% during the upriver migration to the spawning areas (Fig. 6). This reduced, but did not eliminate the between population differences in relative ovarian fat content (St. Johns 0.23 g/g somatic tissue; Connecticut 0.30 g/g somatic tissue).

Significant sex-related differences in viscera fat content at the time of river entry occurred in both populations. Connecticut males, despite their smaller size, had viscera fat reserves 300% higher than those of females (Fig. 6). Between sex differences in Florida shad were less extreme; at the river mouth male viscera fat content was ~40% higher than that of females. Viscera fat levels declined rapidly in both populations during upriver movement. However, during the postspawning period in the river, little change occurred (Fig. 6).

Obvious sex-related differences in the somatic fat content of Florida shad existed at river entry. Despite their generally smaller body size, males began the migration with absolute somatic fat reserves approximately equal to those of females (Fig. 6). In contrast, the somatic fat reserves of Connecticut females at initiation of the river migration was greater than that of males, as would be expected from the larger relative body size of the females.

A rapid decline in somatic fat content occurred during the upriver movement of shad in both populations (Fig. 6). Males and females in both populations consumed 50−60% of their somatic fat reserves to reach the spawning grounds. Postspawning losses by all shad were small in comparison to prespawning losses. Connecticut males utilized ~10% of their somatic fat reserves during the seaward migration. Florida males utilized considerably more fat (37%) during and/or immediately after spawning. Postspawning fat expenditures by Connecticut River females were minimal. Females of the St. Johns River population, in contrast, consumed 35% of their initial fat reserves during and/or immediately after spawning (Fig. 6).

Somatic tissues were the most important source of fat during the migrations of both the Florida and Connecticut populations, supplying up to 150 g for metabolic purposes. In comparison, a maximum of 12 g of fat was utilized from viscera sources.

Gonad protein levels exceeded gonad fat levels by an approximate factor of 10 in both populations. Trends in the protein content of the testes and ovary were very similar to those described for fat (Fig. 7), as were the relative quantities of protein in the gonads (per unit somatic weight) in the two populations. Ovarian protein was relatively constant during the upriver migration in the Connecticut population but increased steadily in the St. Johns shad.

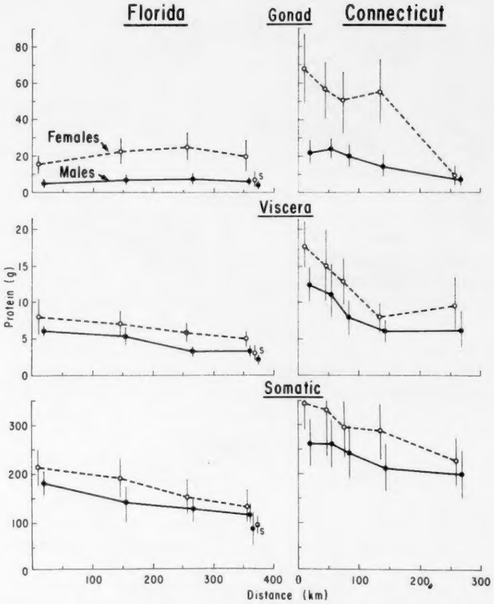

FIG. 7. Tissue protein content of standard shad at various stages of the freshwater migration (S, St. Johns River spent shad).

The viscera protein content of shad in both the St. Johns and Connecticut River populations declined during the upriver migration. Little or no decrease occurred after spawning. In Connecticut, the trends in initial protein depletion were similar in both sexes; the viscera protein levels declined by ~45% (Fig. 7). No decline occurred after spawning. The reduction in viscera protein in Florida shad was more gradual during upriver movement. The proportional losses of Florida migrants were greater (50% for males; 75% for females) than those of Connecticut shad, but the absolute reduction in protein content of 3 g over 370 km of river travel was far less than that experienced by Connecticut shad (6 and 10 g for males and females, respectively) over a shorter migration distance. After spawning, the viscera protein of Florida males and females decreased an additional 17 and 25%, respectively.

Somatic protein declined steadily throughout the migration as did somatic fat. The rate of protein use was greatest during the prespawning phase. Connecticut River males and females consumed ~27 and 40%, respectively, of their initial somatic protein reserves to reach the spawning grounds and to spawn. Somatic protein, like somatic fat, declined rapidly in the Florida population (♂ 15%; ♀ 19%) at or near the time of spawning.

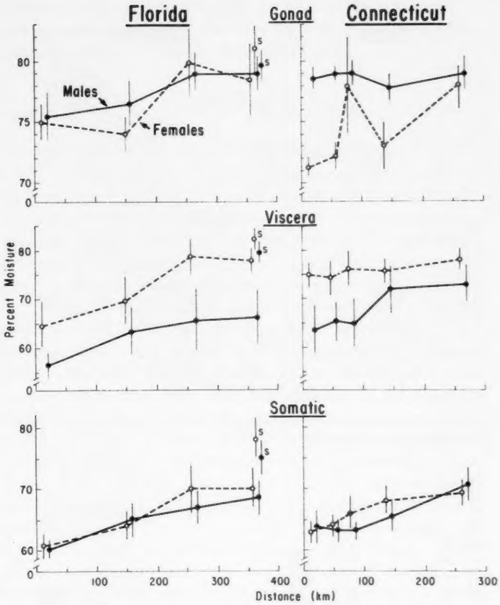

FIG. 8. Tissue moisture content of standard shad at various stages of the freshwater migration (S. St. Johns River spent shad).

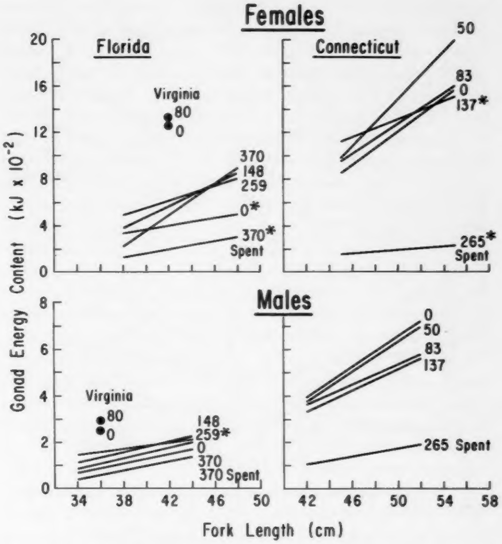

FIG. 9. Gonad energy/body length relationships at various stages of the freshwater migration.

The moisture content of all tissues studied increased throughout the migration in all rivers (Fig. 8). With the exception of the gonads these changes in tissue moisture were inverse, and directly proportional to, changes in tissue fat levels. Changes in gonad moisture content appear to be more related to ripening of the fascicules of ova.

ENERGY DYNAMICS

A marked increase in ovarian energy content occurred during the upriver migration of female shad from the St. Johns River population (Fig. 9). This increase was greatest in large females whose gonads were least developed at river entry (Fig. 2). The average increase in ovarian energy content during the first 259 km of the freshwater migration was 320 kJ (65%) for females 48 cm in length and 160 kJ (47%) for females 38 cm in length. A significant ($P < 0.01$) increase in the slope of the ovarian energy/length relationship at km 370 suggested that smaller females, whose gonads were more developed, spawned first. No significant change in testicular energy occurred prior to spawning.

The gonad energy content of Connecticut River shad, which were more mature than those of the St. Johns population at river entry (Fig. 2), did not change significantly during the upriver migration. No size-related trends in spawning times were apparent in the data (Fig. 9).

The energy content of the ovaries of Virginia shad which, like those of Connecticut females, were at an advanced stage of maturity at river entry (Fig. 2) did not change significantly (Fig. 9). The energy content of the testes of Virginia shad, which were intermediate in maturity to the gonads of Connecticut and Florida migrants did, however, experience a small (30 kJ) but significant ($P < 0.01$) increase in energy content.

The per-unit-length viscera energy reserves of St. Johns River shad were greater than those of Connecticut River females at river entry ($P < 0.01$). No significant difference remained at the spawning areas ($P > 0.5$). This fact explains the higher proportional utilization (Florida 75%; Connecticut 60%) of initial viscera reserves by St. Johns females (Fig. 10). The larger St. Johns River females, the ovaries of which had the lowest energy content at river entry, possessed viscera energy reserves up to 198% (630 kJ) greater than those of smaller females in the same population. These larger females experienced a more rapid reduction in viscera reserves during the early phase (km 0–148) of the freshwater migration than did their smaller counterparts (600 vs. 100 kJ, respectively; Fig. 10). This change paralleled the rapid increase in ovarian energy content (360 kJ) in these fish over the same phase of the migration (Fig. 9) suggesting a transfer of energy from the viscera to the ovaries. No such reciprocal changes in tissue energy content occurred in St. Johns River males, although larger males, too, experienced a more rapid decline in viscera reserves, notably during the latter half of the upriver migration. No size-dependent differences in viscera energy use occurred in Connecticut River shad. Males in both populations used a greater proportion of their total viscera reserves to reach the spawning grounds (Connecticut 65%; Florida 90%) than did females (Connecticut 60%; Florida 80%) (Fig. 10). York River shad entered the river with viscera energy reserves equivalent to those of Connecticut River fish. Depletion of viscera reserves during the upriver migration (♂ 22%; ♀ 30%) was, however, much less than that

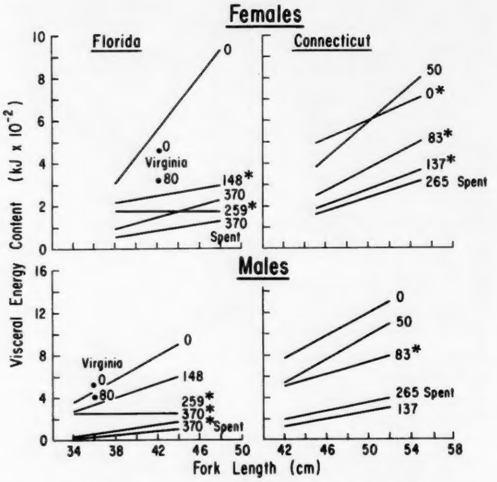

FIG. 10. Viscera energy/body length relationships at various stages of the freshwater migration.

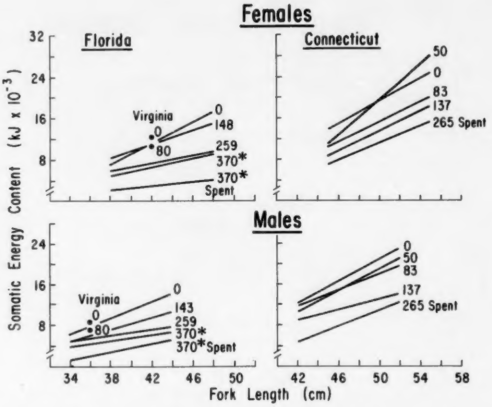

FIG. 11. Somatic energy/body length relationships at various stages of the freshwater migration.

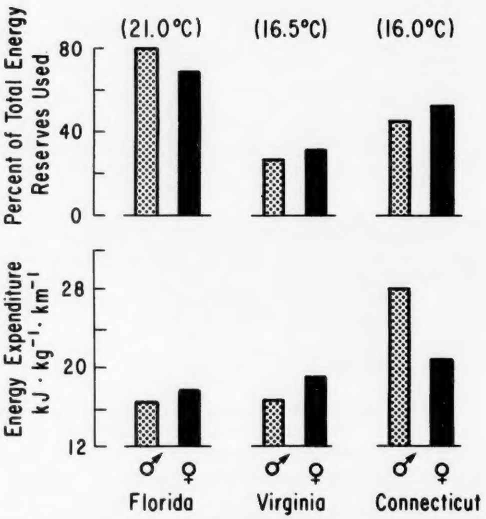

FIG. 12. Absolute and relative energy expenditure during the freshwater migration and average water temperature during the migration.

experienced by St. Johns and Connecticut River shad (Fig. 10).

Shad of both sexes in the St. Johns and Connecticut River populations began their freshwater migrations with equivalent per-unit-length somatic energy reserves ($P > 0.05$). However, because of differences in the average size of individuals in the two populations the absolute somatic energy reserves of the standard Connecticut River fish exceeded those of the standard St. Johns River fish by 32% (440 kJ) and 55% (940 kJ) in the case of males and females, respectively. The somatic energy reserves of York River shad were comparable to those of the St. Johns River fish (Fig. 11).

The proportion of the somatic energy resources used to fuel the migration to the spawning grounds and for spawning was greatest in the St. Johns River fish (♂ 65%, ♀ 80%), intermediate for those of the Connecticut River (♂ 48%, ♀ 45%), and least for York River shad (13%) (Fig. 11). The proportional energy expenditure experienced by the standard St. Johns River shad to complete the upriver migration and to spawn (65–80%) was greater than the energy expenditure by Connecticut River shad during the complete migration to the spawning migration and back to the sea (maximum = 60%). The 15–30% decrease in somatic reserves during the seaward migration by Connecticut River shad represented the total cost of this movement since no change in viscera energy stores occurred during this phase of the migration. Absolute somatic energy use by the standard St. Johns River shad to complete the upriver migration and spawn (♂ 7400 kJ, ♀ 9200 kJ) was equivalent to the absolute somatic energy expenditure by standard Connecticut River shad to complete the entire freshwater spawning migration (♂ 9600 kJ, ♀ 8600 kJ).

ENERGY COST OF MIGRATION

Shad of the St. Johns River population had the lowest energy expenditure · kg body weight^{-1} · km^{-1}. Shad of the York River population were similar to those of the St. Johns population in this regard, while Connecticut River fish had a significantly higher energy expenditure per unit distance (Fig. 12).

The proportion of total viscera and somatic energy utilized to complete the migration was greatest in Florida, intermediate in Connecticut, and least in Virginia (Fig. 12). Losses experienced by Virginia migrants (26–32%) were estimated by doubling the proportional losses associated with upstream migration. The assumption that downstream costs equalled those associated with the upriver migration in Virginia provides a maximum estimate of total energy expenditures required to complete the migration since maximal energy expenditure after spawning did not exceed 60% of the upstream losses in Florida and Connecticut.

Discussion

Adult shad do not feed in freshwater (Atkinson 1951). There is, therefore, a rapid decline in energy reserves, accumulated at sea, during the freshwater phase of the spawning migration. This rapid and extensive use of energy reserves is reflected in the extreme weight loss suffered by shad during the migration (Chittenden 1969; Leggett 1972; this study). The use of tissue weight change as a morphological index for the evaluation of the energy dynamics of fish populations has been criticized in the past (Shulman 1974; Gerking 1952). We found, however, that changes in tissue mass closely paralleled changes in tissue energy content and for this reason tissue weight change can serve as a reliable index of the extent of energy utilization in shad.

The important variables influencing the rate and extent of energy utilization by shad are temperature (Glebe and Leggett 1981), body size, sex, water current velocity, stage of gonad development prior to entry into freshwater, and distance to spawning areas. The large number of contributing factors resulted in high variability in measures of tissue weight change and energy depletion both within and between the Connecticut, York, and St. Johns River populations. There were, however, important differences in the patterns of tissue weight dynamics and energy use in the three populations which appear to be related to individual survival and, ultimately, the life history characteristics of the populations.

GONAD DEVELOPMENT

There was a clear latitudinal trend in the state of development of the gonads at the time of entry into the rivers. Gonads of the St. Johns River shad were the least developed, those of the Connecticut River the most fully developed, while gonads of the Virginia population were intermediate in condition. In the St. Johns River population gonad weight and energy content increased in both females and in males during upriver migration to the spawning grounds. This energy, averaging 220 kJ in females and 30 kJ in males, was drawn from somatic and viscera reserves. The transfer of energy to the gonads of York River shad during the migration was smaller, averaging <30 kJ. Connecticut River shad exhibited virtually no change in gonad weight or gonad energy content during the migration to the spawning grounds. In this group gonad development was completed at sea.

The positive relationship between gonad development at the time of river entry and latitude of the home river leads us to hypothesize that gonad development begins at approximately the same time in all Atlantic Coast stocks. Shad from all Atlantic Coast populations intermingle and migrate together along the Atlantic Coast in response to seasonal changes in the location of preferred water temperatures (Leggett and Whitney 1972; Neaves and Depres 1979). Consequently, shad from all Atlantic Coast populations experience similar feeding and environmental conditions while at sea. Assuming synchronous initiation of gonad development, possibly in response to increasing day length (Baggerman 1957), Florida shad would arrive at their home river with their gonads in an early stage of development relative to gonad development in shad of more northern populations which enter their home rivers to spawn later in the spring. The orderly south−north sequence of river entry (Walburg and Nichols 1967; Leggett and Whitney 1972) would produce the latitudinal trend observed. This is a proximate explanation only. Egg and early larval survival in shad is highly temperature-dependent and spawning is strongly keyed to the optimum temperature which is similar at all latitudes (Bradford et al. 1968; Leggett and Whitney 1972; Marcy 1976; Leggett 1977). Shoubridge and Leggett (1978) have proposed that the low ovarian energy content characteristic of St. Johns River shad is an adaptation making the final ripening of the eggs, which is normally temperature-dependent in shad (Walburg and Nichols 1967), independent of the thermal regime of the river. The delayed transfer of energy from somatic and viscera reserves to the ovary in southern populations could serve to prevent ripening of the eggs until the spawning grounds are reached. The earlier spawning exhibited by smaller St. Johns River females, whose gonads were more fully developed at river entry, may also be related to this phenomenon. In more northern rivers, where temperatures during the initial phase of the migration are below the threshold for final ripening, the effect of temperature on egg ripening is delayed until later in the migration thereby obviating this problem.

Energy utilized for the development of the gonads while at sea is diverted from somatic and viscera reserves. We base this conclusion on our finding that the proportional viscera reserves of large St. Johns River females, whose ovaries were poorly supplied with energy at river entry, were greater than those of smaller females, whose gonads were more fully developed. Similarly, the somatic and viscera reserves of female Connecticut River shad, whose ovaries were fully supplied with energy at the time of river entry, were proportionally lower than those of female Florida shad, whose ovaries were incompletely developed at river entry. This occurred in spite of the longer period of ocean feeding in Connecticut River shad, made possible by their later entry into their respective home rivers to spawn. This fact makes direct comparisons of the amount of energy diverted from viscera and somatic reserves to gonad impossible. Similar patterns of energy transfer have been reported for herrring (*Clupea harengus*, Blaxter and Holliday 1963; Channon and Saby 1932) and *Sardinia* (Clupeidae, Hickling 1945).

In shad, the total energy content of the testes of the standard males is <40% that of the ovaries of the standard female. The relative energy cost of gonad development at sea is reflected in the viscera stores of the two sexes, males in all populations having higher proportional somatic and viscera energy reserves than do females at the time of river entry. The difference in the absolute energy content of the ovaries and testes of fish of equivalent sizes was similar (±10%) to the difference in the absolute energy content of the viscera. The testes of York and Connecticut River shad are virtually mature at the time of river entry, while those of St. Johns River males require a relatively small transfer of energy (42 kJ for the average (40 cm) shad in the population) during the migration. This may explain the absence of a latitudinal trend in somatic and viscera energy reserves in males sampled at the mouths of their respective rivers.

MIGRATION ENERGETICS

The energy cost of migration to the spawning grounds differed between populations and was dependent on body size, current velocity, and distance to spawning areas. In the Connecticut River, male shad expended more energy (29.3 $kJ \cdot kg^{-1} \cdot km^{-1}$ of upstream displacement) than did females (20.9 $kJ \cdot kg^{-1} \cdot km^{-1}$ of upstream displacement). This appears to result primarily from the difference in average size of males and females since conditions experienced during the migration and the average rates of upriver migration were similar for the two groups. Size-related differences in metabolic rates are common in fish and appear to result from both physiological and hydrodynamic factors (Winberg 1956; Fry 1971; Brett and Glass 1973; Webb 1975; Trump and Leggett 1980; Fig. 14). The higher energy cost of migration in smaller shad is consistent with the results of these theoretical and empirical studies.

In the St. Johns River population, somatic and viscera energy losses were higher in females than in males (18.4 kJ and 17.2 $kJ \cdot kg^{-1} \cdot km^{-1}$ of upstream displacement respectively for standard shad) and are higher for large females than for small females. This disparity in the typical body-size, swimming-efficiency relationship, appears to result from differences in the rate of transfer of energy to the gonads within and between populations. Females of the St. Johns population enter the river at earlier stages of gonad development and large females are considerably less mature than smaller fish in the St. Johns population. The resulting transfer of energy reserves from somatic to gonad tissue during the freshwater migration produces a greater rate of somatic and viscera energy loss in females than males and a higher loss among large females relative to smaller individuals of the same sex. The absolute energy transfer to the ovary in the standard St. Johns River female averaged 240 kJ or 0.93 kJ/km over the first 259 km of the migration to the lower limit of the spawning area. This closely approximates the 1.2 kJ/km difference in energy expenditure between sexes in the population. The somatic energy loss for gonad development thus overrides the metabolic advantage of size. Idler and Clemens (1959) reported similar trends in energy utilization by sockeye salmon. Female sockeye expended 20% more energy per kilometre than males. They attributed this disparity to the greater demands of gonad maturation in females.

The higher energy utilization of large vs. small males in both the Florida and Connecticut populations is inconsistent with the general pattern of increasing locomotory efficiency with increasing size. Since males do not transfer significant quantities of energy to the testes during migration, large individuals should have a metabolic advantage but do not. The burden of spawning for large males may be greater than that indicated solely by changes in gonad energy content. Hickling (1930) reported the cost of spawning in male *Merluccius merluccius* doubles for every 10 cm increase in body length. The reasons for this disparity are, however, unknown.

The average cost of the migration ($J \cdot kg^{-1} \cdot km^{-1}$) for the Florida and Virginia population was significantly less than that recorded for the Connecticut population. Reduced current velocities in the St. Johns and York Rivers, indicated by lower angular displacement to the spawning areas (~5 m deg compared to 20 m deg in the Connecticut River) are believed to account, in part, for this difference. The reduced amplitude of tidal-induced oscillations in current velocity in the St. Johns River, relative to those of the Connecticut River, should also reduce the energetic costs of migration there (Leggett and Trump 1978; Trump and Leggett 1980).

In spite of the lower energy expenditure/unit distance travelled, shad from the St. Johns River population experienced significantly greater total reductions in body weight (20−50%) and energy reserves (70−80%) than did Connecticut and York River shad. Connecticut River shad experienced body weight losses of 25−35% and utilized 40−60% of their combined somatic and viscera energy reserves during the complete freshwater migration from the sea to the spawning grounds and back to the sea. Extrapolation of the energy utilized by York River shad to reach the spawning area indicates these fish utilize ~32% of their total energy reserves to complete the freshwater migration. The higher total energy expenditure in Florida results from the combination of greater energy transfer to the gonads during the freshwater migration and the longer freshwater migration required to reach the spawning areas in the St. Johns River.

LIFE HISTORY CONSEQUENCES

The high weight loss and energy expenditure experienced by the St. Johns River shad during the freshwater migration to the spawning area precludes a successful return migration to the sea. Energy expenditures of similar magnitude have been reported for Pacific salmon (Fig. 13, Greene 1926; Pentegov et al. 1927; Idler and Clemens 1959) which, like St. Johns River shad, suffer complete postspawning mortality (Leggett and Carscadden 1978). At the proximate level, therefore, the combined energetic costs of gonad development and the freshwater migration are the reason for the semelparous reproductive character of the Florida shad population. Even by delaying maturity, thereby increasing the average body size (and energy reserves) to levels equivalent to those of the Connecticut stock, and by reducing the energy allocated to the gonad to that characteristic of northern populations, Florida shad would experience lethal energy expenditures preventing a successful return migration to the sea. However, the lack of repeat spawners in other southern rivers where shorter migration distances prevail (Ogeechee R., Edisto R.), and the reduced frequency of repeat reproduction in populations spawning in rivers located at intermediate latitudes (Leggett and Carscadden 1978) where shorter migration distances (Walburg and Nichols 1967) should permit a return migration to sea, indicates that, contrary to earlier predictions (Leggett 1972), energy constraints are not the principal regulators of latitudinal differences in the life history characteristics of shad populations.

It now appears that it is through, rather than because of, differences in energy allocation and use that latitudinal differences in life history strategies are acheived. Shoubridge and Leggett (1978) have shown that the probability of the occurrence of suboptimal temperature conditions during the main reproductive period for shad varies inversely with the latitude

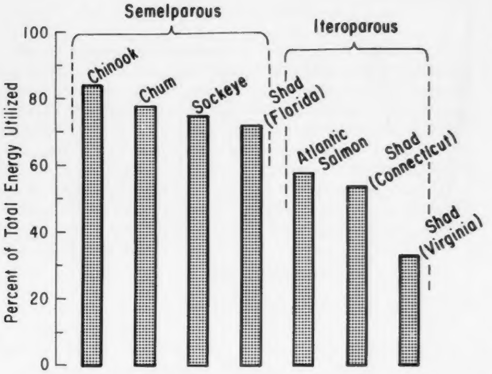

FIG. 13. Energy use by representative single (semelparous) and repeat (iteroparous) spawning anadromous fishes during the freshwater migration. Atlantic salmon, Greene 1926; Chinook, Greene 1926; chum, Pentegov et al. 1927; sockeye, Idler and Clemens 1959.

of the home rivers; southern rivers are predictably good, northern rivers are unpredictable. Since water temperatures during egg and early larval development significantly influence spawning success, year-class strength, and recruitment (Marcy 1976; Leggett 1977; Shoubridge and Leggett 1978), annual density-independent variations in juvenile mortality should be small in the south relative to the north. In addition, year-to-year fluctuations in juvenile mortality exceed that of adults by ~4:1 (Shoubridge and Leggett 1978). Current life history strategy theory (Stearns 1976) predicts that under such circumstances natural selection would favor individuals exhibiting shorter life, fewer reproductive acts, higher fecundity, and larger reproductive effort in the south and the opposite condition in the north. This is precisely the pattern reported for southern shad populations by Leggett and Carscadden (1978). Hence, at the ultimate level, it appears that differences in the predictability of the reproductive environment, through its influence on juvenile mortality, is the principal regulator of latitudinal differences in life history strategies in shad. When viewed from this perspective, interpopulation differences in energy allocation to migration vs. reproduction in shad must be seen as a consequence (rather than a cause) of the bet hedging (Stearns 1976) life history patterns observed.

The idea of the differential allocation of energy resources to various functions in order to maximize fitness in environments whose characteristics change in an orderly way over the species' range is not new, although ours may be the first empirical demonstration of the phenomenon. Cody (1966), Holgate (1967), Gadgil and Bossert (1970), Charnov and Krebs (1973), and Mountford (1973) have all inferred from a variety of perspectives that optimum life history strategies may be achieved by allocating the limited energy resources available to an individual to reproduction, growth, and maintenance in a way that maximizes that indivudual's contribution to future genotypes.

In the case of shad the important question is the way in which available energy resources are partitioned between migration (adult survival) and reproduction. To begin, it should be noted that even in populations spawning in northern rivers where adult postspawning survival is high the energy allocation to migration is close to the minimum required to achieve this high adult survivorship which dampens the effects of variable juvenile survival on population size (Murphy 1968; Schaffer 1974) and stability (Ricker 1954, 1958; Beverton and Holt 1957). In the Connecticut River, for example, the increased energy expenditure experienced by shad migrating late in the spawning run (Glebe and Leggett 1981) when water temperatures are higher than the average experienced by the population are positively correlated with higher postspawning mortality in these fish relative to those migrating early or at the peak of the run (Leggett 1969). Overall adult mortality is also positively correlated to the thermal regime of the river during the migration, being higher in years when the water temperature during the migration is higher than average ($F_{1,7} = 7.523$, $P < 0.05$; Shoubridge and Leggett 1978). These findings suggest that even in highly iteroparous northern populations the energy allocated to reproductive products is close to the maximum possible given the constraints placed on life history strategies by the reproductive environment occupied.

There was, however, a significant difference in both the relative and absolute allocation of energy to the gonads in the semelparous St. Johns River and iteroparous Connecticut River shad. A quantitative evaluation of this difference requires some interpretation of the changes in tissue energy occurring in St. Johns River fish during the migration. Leggett and Carscadden (1978), using data reported here, have proposed that the rapid decline in somatic energy reserves experienced by the St. Johns River shad at the spawning grounds represents a final transfer of energy to the ovum immediately prior to spawning. Three factors argue in support of this hypothesis: first, the magnitude of this decline in energy reserves is too great to be attributed to maintenance requirements alone (~25% of initial somatic reserves (3300 kJ) as compared with a 50% reduction in initial somatic reserves to complete the 370-km upriver migration to the spawning grounds); secondly, if all ova were fully developed prior to spawning, as occurs in the Connecticut River, the volume of eggs would exceed the capacity of the body cavity — sequential development of the ova is thus a necessity; and, finally, this transfer of energy to the gonad on the spawning grounds simply represents a continuation of a process known to be ongoing throughout the spawning migration. Their analysis led to the conclusion that the average energy content/ovum at the time of spawning would be ~5.8 J which compares with 5.4 J/ovum for the average Connecticut River female. In view of the approximations required in the calculation we do not consider this difference to be biologically meaningful. The similarity of these two measures is logical since the average temperature during incubation in all rivers is similar and there is, therefore, no reason to expect the threefold lower energy content/ovum in the St. Johns River population that would prevail if additional energy were not transferred to the ovary immediately prior to spawning.

These calculations indicate that the absolute energy allocated to eggs by the average female in the St. Johns River population (2300 kJ) is 1.6 times the energy allocated to eggs by Connecticut River females (1440 kJ). Proportionally, St.

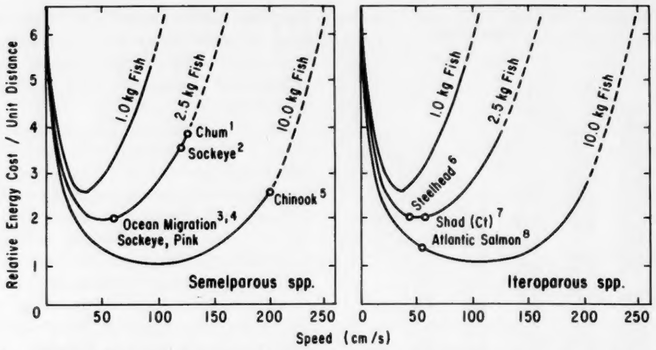

FIG. 14. Speed efficiency curves for fish of various sizes and reported migration speeds of seven anadromous fish species during freshwater and ocean migrations. (1) Pentegov et al. 1928, (2) Idler and Clemens 1959, (3) Stasko et al. 1973, (4) Madison et al. 1973, (5) Greene 1926, (6) Monan et al. 1970, (7) Leggett 1976, (8) Hayes 1953.

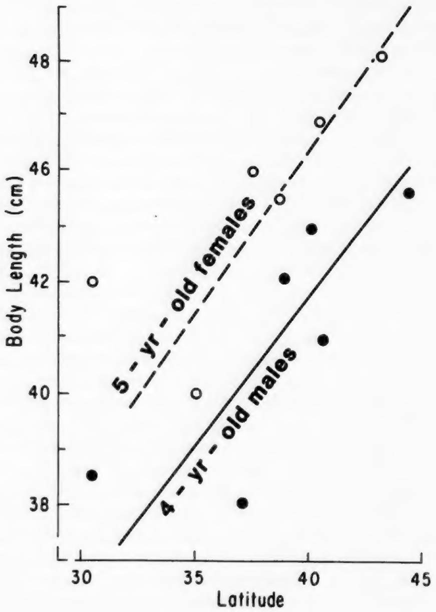

FIG. 15. Mean shad body size at maturity vs. latitude of the home river. Data from Carlander 1969; Chittenden 1969; this study.

Johns River females allocate 16% of their total body energy reserves to reproductive products whereas Connecticut River shad allocate only 7% of their energy reserves for this purpose, the balance being used to provide the migration energy required to ensure a successful return to the sea.

Examination of the relationship between energy expenditure during the spawning migration and postspawning survival suggests there may be a common pattern among anadromous species (Fig. 13). In all cases for which data could be found, iteroparous species consumed less than 60% of their total energy reserves during the migration. Atlantic salmon, which have a low postspawning survival (average = 6% for 24 North American populations, Schaffer and Elson 1975) approach this value. Semelparous species, in contrast, all exhibited energy expenditures associated with migration and spawning that exceeded 70% of the total energy available.

SWIM SPEEDS AND MIGRATION EFFICIENCY

In addition to the differences in energy allocation and use between iteroparous and semelparous migrants, there appears, also, to be a basic difference in freshwater swim speeds and migration efficiency between the two. Semelparous species, which allocate a higher proportion of total reserves to reproduction at the expense of adult survival, swim at speeds close to the maximum sustained speed and at which energy expenditure per unit distance is substantially higher than the optimum (Fig. 14). These higher speeds would minimize the time spent in freshwater prior to spawning, but result in higher rates of use of energy reserves, Iteroparous species, in contrast, swim at speeds close to the optimum, thereby minimizing the energy cost of migration and improving the probability of a successful return migration to the sea (Fig. 14). Interestingly, semelparous species appear to swim at speeds close to the optimum while at sea, thereby maximizing the net energy gain from feeding.

It is worthy of note, too, that the range of swimming speeds over which energy cost/unit distance is optimized is a function of body size, larger fish having a greater range of speeds over which changes in rates of energy expenditure and hence the absolute energy cost of migration, are small. This fact may help to explain the widespread positive relationship between body size and river harshness in Atlantic salmon (Schaffer and Elson 1975) and the larger body size of mature shad in northern rivers in which the river gradient and seasonal runoff combine to increase current velocities relative to those experienced in the south during the spawning migration (Fig. 15).

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Deep-water eelpouts, Zoarcidae, from Arctic Canada and Alaska

DON E. MCALLISTER

National Museum of Natural Sciences, National Museums of Canada, Ottawa, Ont. K1V 7B2

M. ERIC ANDERSON

Virginia Institute of Marine Science, Gloucester Point, VA 23062, USA

AND J. G. HUNTER

Arctic Biological Station, Fisheries and Oceans Canada, Ste-Anne-de-Bellvue, Que. H9X 3R4

MCALLISTER, D. E., M. E. ANDERSON, AND J. G. HUNTER. 1981. Deep-water eelpouts, Zoarcidae, from Arctic Canada and Alaska. Can. J. Fish. Aquat. Sci. 38: 821–839.

Lycodes sagittarius and *Gymnelus retrodorsalis* are reported from Canada for the first time; *L. eudipleurostictus* and *L. squamiventer* are reported from Alaska for the first time, extending their known ranges 2400 and 4000 km, respectively; the known range of *L. rossi* is extended over 1300 km eastward, *L. seminudus* 1900 km southwestward, and the known range of *L. frigidus* over 6500 km eastward. Descriptions and synonymies are presented. Keys to these and other species of Zoarcidae from Canada are provided.

Key words: Zoarcidae, Arctic, Canada, *Gymnelus*, *Lycodes*, eelpouts, Alaska

MCALLISTER, D. E., M. E. ANDERSON, AND J. G. HUNTER. 1981. Deep-water eelpouts, Zoarcidae, from Arctic Canada and Alaska. Can. J. Fish. Aquat. Sci. 38: 821–839.

Lycodes sagittarius et *Gymnelus retrodorsalis* sont homologués au Canada pour la première fois, tandis que *L. eudipleurostictus* et *L. squamiventer* le sont en Alaska, également pour la première fois, leur aire de distribution connue étant ainsi élargie de 2400 et 4000 km, respectivement. Celle de *L. rossi* est ainsi accure de plus de 1300 km vers l'est, celle de *L. seminudus* de 1900 km vers le sud-ouest et celle de *L. frigidus* de plus de 6500 km vers l'est. Les descriptions et synonymies sont fournies. Le présent article contient des clefs d'identification de ces espèces ainsi que d'autres espèces de Zoarcidae du Canada.

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Dedicated to Anatoly P. Andriashev, Zoological Institute, Leningrad, on the occasion of his 70th yr, in tribute to his work on Arctic fishes, especially his contributions on the family Zoarcidae.

THE deep waters of Arctic Canada and Arctic Alaska have been little explored. About a dozen trawl haul stations in depths of up to 464 m have revealed seven species of eelpouts new to, or little known in, these waters. This paper describes these species, introduces new diagnostic characters, and records extensions to their known range.

The format for the species accounts is that used by Able and McAllister (1980) in a revision of Arctic Canadian *Liparis*. Two new Alaskan records from the Beaufort Sea and one from the Chukchi Sea are included for convenience and because they will probably be found in Canadian waters when more deep-water samples are made. Diagnoses and keys apply to Arctic Canadian and Alaskan populations only. Life history sections are included only for those species currently known from Canada. These accounts are contributions towards a proposed book on the Arctic Canadian ichthyofauna.

Materials and Methods

Owing to variation in characters with locality, size, sex, identification of zoarcids can be difficult. Scalation and lateral lines are incomplete, for example, in small specimens. Poor preservation can compound the difficulty, defacing the color pattern, obscuring the tiny neuromasts and scales with mucus, and contorting specimens making measurements difficult. Killing the specimens quickly in Formalin and preserving the specimens straight with plenty of space in the container will help greatly with identification of, and research on, zoarcids.

Almost all the material studied is in the National Museum of Natural Sciences, National Museums of Canada, Ottawa, much of it collected by the Arctic Biological Station,

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Fisheries and Oceans Canada. Species descriptions are based on Canadian material, except for *Lycodes frigidus*, *L. squamiventer*, and *L. eudipleurostictus*.

Measurements and counts were taken following methods described by Andriashev (1954), pore counts by Gosztonyi (1977). Sample sizes of meristic characters are given in square brackets following the ranges.

In the descriptions the following abbreviations are used: D, dorsal fin rays; C, caudal fin rays; A, anal fin rays; P, pectoral fin rays; Br, branchiostegals; GR, gill rakers; Abd. vert., abdominal vertebrae; Caud. vert., caudal vertebrae; T. vert., total vertebrae; Pyl. caeca, pyloric caeca; N.W.T., Northwest Territories; Y.T., Yukon Territory; and TL, total length. For museums we use the following abbreviations: BMNH, British Museum (Natural History); MCZ, Museum of Comparative Zoology, Harvard; MNHN, Musée national d'Histoire naturelle, Paris; NMC, National Museum of Natural Sciences, National Museums of Canada, Ottawa; USNM, United States National Museum, Washington; ZIL, Zoological Institute, Leningrad; ZMUC, Zoological Museum, University of Copenhagen; ZMUO, Zoological Museum, Univesity of Oslo.

Provisional Key to the Zoarcidae of Arctic Canada

All species known from Arctic Canada and three Arctic Alaska species expected in Canadian waters are included.

1 Pelvic fins absent; pectoral rays 9−14; gill opening not reaching below ventral edge of pectoral fin base .... Gymnelinae 2

Pelvic fins present; pectoral rays 15−22; gill opening extending down to or below ventral edge of pectoral fin base .... Lycodinae 4

2 Vertebrae 92−100; body and tail unbanded, or with bands on tail always descending below lateral line 3

Vertebrae 87−91; body and tail with 12−15 dark bands; those on tail descending only to lateral line .... *Gymnelus hemifasciatus* Andriashev, 1937

Canadian records to be published elsewhere by M.E.A.

3 Dorsal fin origin above pectoral fin; at most two dorsal fin pterygiophores at front of fin without a dorsal ray; height of pectoral fin base 39−60% of the length of fin; predorsal length $\frac{1}{5}$ or less of TL .... *Gymnelus viridis* (Fabricius, 1780)/fish doctor/unerak

Dorsal fin origin behind end of pectoral fin; first 3−14 dorsal fin pterygiophores without rays; height of pectoral fin base 25−39% of the length of the fin; predorsal length $\frac{1}{4}$ or more of TL .... *Gymnelus retrodorsalis* Le Danois, 1913/aurora pout/unerak aurora

4 Lateral line (consisting of small pale neuromasts) descends or dips below midline on abdomen; peritoneum dark; preanal length short, 37−46% TL; scales on body and vertical fins of adults .... *long-tailed Lycodes* 5

Lateral line mediolateral on abdomen: peritoneum pale except in *L. seminudus*; preanal length long, 45−55% TL; scales present or absent, but never on vertical fins .... short-tailed *Lycodes* 8

5 Lateral line single 6

Lateral line double, ventrolateral, and mediolateral 7

6 Lateral line bowed down on abdomen, returning to midlateral position above anal fin origin; pectoral rays 16−18; 20−27 scales across body at level of anal fin origin .... *Lycodes sagittarius* McAllister, 1976/archer eelpout/lycode à arc

Lateral line descends below midline on abdomen and continues just above anal fin to tip of tail; pectoral rays 19−21; 42−48 scales across body at level of anal fin origin .... *Lycodes frigidus* Collett, 1878/cold water eelpout/lycode glaciale
Not yet known from Canadian Arctic Waters

7 Pectoral fin well rounded, without fork; anal rays 79−87, pectoral rays (17)18−20(21); vertebrae 96−104 8

Pectoral fin shallowly forked; anal rays 91−96, pectoral rays 20−22(23); vertebrae 106−112 .... *Lycodes eudipleurostictus* Jensen, 1902/doubleline eelpout/lycode à deux lignes
Not yet known from Canadian Arctic waters

8 Belly naked on underside; body and dorsal fin usually with 9−12 dark bands, but often monotone in large specimens .... *Lycodes pallidus* Collet, 1878/pale eelpout/lycode pâle

Belly scaled on underside; body monotone, no banding on dorsal fin .... *Lycodes squamiventer* Jensen, 1904/scaly eelpout/lycode écailleuse
Not yet known from Canadian Arctic waters

9 Scales at least on tail region 10

Without scales 13

10 Peritoneum pale; gill opening reaching to but not below edge of pectoral fin base; pectoral fin length 12−15% TL 11

Peritoneum dusky; gill opening reaching below base of pectoral fin; pectoral fin length 9−12% TL .... *Lycodes seminudus* Reinhardt, 1837/longear eelpout/lycode à oreilles

11 Seven to 10 dark crossbands on body which cross and may merge below the midline; pectoral rays 19−21; 93−99 vertebrae 12

Five to six dark saddles with well-defined borders contrasting strongly with narrow light areas between; unpigmented areas above anal fin; pectoral rays 17−18 (scaled specimens key out here); 90−92 vertebrae .... *Lycodes mucosus* Richardson, 1855/saddled eelpout/lycode à selles

12 Dark crossbands on body reticulate on large specimens; pectoral rays 19−21; 93−96 vertebrae .... *Lycodes reticulatus* Reinhardt, 1835/Arctic eelpout/lycode arctique

Dark crossbands never reticulated; pectoral rays usually 18−19; 98−99 vertebrae .... *Lycodes rossi* Malmgren, 1864/threespot eelpout/lycode à trois taches

13 Vomerine teeth 2−7, pointed; saddlelike marks or bars on back not V-shaped; 90−94 vertebrae 14

Vomerine teeth 8−14 (fewer in young), round or flat-topped in adults; V-shaped dark bars on back; 99−102 vertebrae .... *Lycodes jugoricus* Knipowitch, 1906/shulupaoluk/lycode plume

14 Five to six dark saddles with well-defined borders on back; pectoral fin 13−15% TL; usually a ridge behind first pore or neuromast on tip of chin (scaleless specimens key out here) .... *Lycodes mucosus* Richardson, 1855/saddled eelpout/lycode à selles

Nine to 11 dark bars with diffuse edges on body; pectoral fin 9−13% TL; without ridge behind first pore or neuromast on tip of chin .... *Lycodes polaris* (Sabine, 1824)/polar eelpout/lycode polaire

Subfamily LYCODINAE

Genus *Lycodes* Reinhardt, 1831

Lycodes Reinhardt, 1831: 18 (type: *Lycodes Vahlii* Reinhardt, by monotypy; gender masculine)

Lycodalepis Bleeker, 1874: 369 (type: *Lycodes mucosus* Richardson, 1855, by original designation)

Furcella Jordan and Evermann, 1896: 480 (type of subgenus: *Lycodes diapterus* Gilbert, 1891, by original designation; preoccupied by *Furcella* Lamark, 1801, in Mollusca)

Lycias Jordan and Evermann, 1898: 2461, 2463, (type of subgenus: *Lycodes seminudus* Reinhardt, by original designation)

Furcimanus Jordan and Evermann, 1898: 2472 (type: *Lycodes diapterus* Gilbert, 1891, by original designation; replacement name for *Furcella* Jordan and Evermann, 1896)

Bergeniana Popov, 1931: 140 (established for *Lycodes uschakovi*, *L. colletti*, *L. lindbergi*, and *L. semenovi*)

Etymology — *Lycodes* from the Greek for wolfish in reference to its supposedly wolfish appearance.

Body moderately elongate; dorsal, anal, and caudal fins continuous, without notches or spinelike rays; lateral line mediolateral and/or ventrolateral; nostril tubular, single; gill openings extending down to just above lower edge of base of pectoral fin or beneath it; upper jaws protrude beyond lower; vomer and palatines with teeth; mental lobes separated by a space (or fused); palatal and mandibular membranes completely reduced; two short pyloric caeca usually present; vertebrae 87−119; vertebral centra symmetrical.

More than 45 species in arctic and temperate seas, 1 in the Southern Hemisphere.

Lycodes rossi
Malmgren, 1865
Fig. 1 and 2

threespot eelpout
lycode à trois taches (f.)

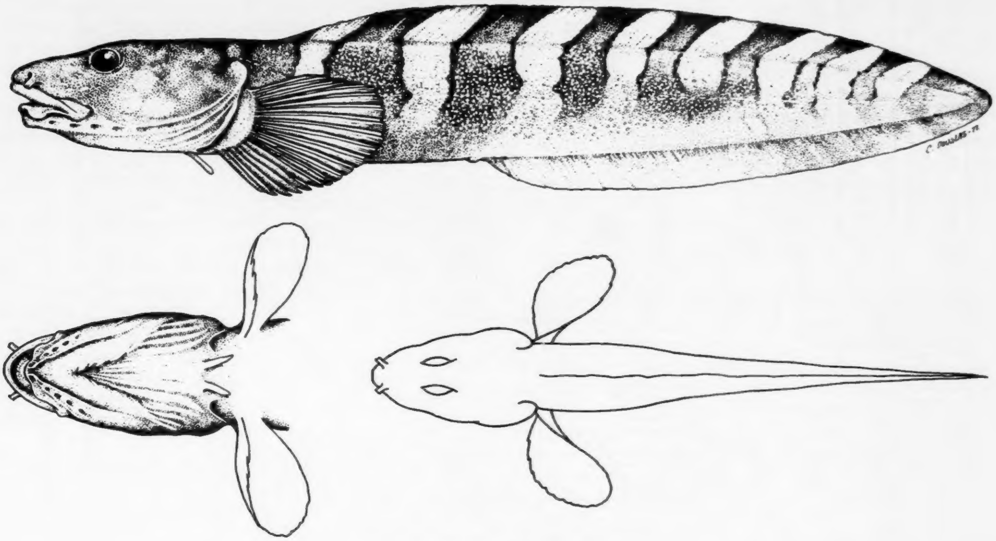

FIG. 1. *Lycodes rossi* (NMC 77-0976).

Synonymy

ORIGINAL DESCRIPTION

Lycodes rossi Malmgren, 1865: 516 (Treurenberg Bay, Spitsbergen, in 5 fathoms (1 fathom = 1.8 m), type 32 mm long in Stockholm Museum).

CANADIAN ARCTIC RECORDS

Lycodes rossi, McAllister 1962: 26 (distr., descr., 7 miles (1 mile = 1.6 km) NE Collinson Head, Herschel Island, Y.T.).

SUPPLEMENTARY REFERENCES

Lycodes rossi, Jensen 1904: 55, pl. VII, fig. 1a (type), b, c, d, e, f, g (synon., descr., taxon., distr., depth); Andriashev 1954: 284 (305 in translation), fig. 155–157, inset 2 (synon., descr., distr., life hist.).

Etymology — *rossi* after Sir James Clark Ross, Arctic explorer and naturalist.

Diagnosis

Differs from other *Lycodes* in the region by the mediolateral lateral line, scales extending forward to midpectoral fin, pectoral rays (17)18–19(20), pectoral length 15% or less TL, body with six to nine light bars with dark edges across dorsal fin and back. Anal rays 74–80, including half the caudal rays.

Description

MERISTICS

Range and sample size for the Canadian Arctic.

D + ½C 91–95 [9]
A + ½C 74–80 [9]
C 9–10 [2]
P (17)18–19(20) [20]
Br 6 [18]
GR 1–3 + 10–12 = (11)12–14 (15) [19]
Abd. vert. 23–25 [10]
Caud. vert. 70–75 [10]
T. vert. 95–98 [10]
Pyl. caeca 2 [19]

Head — Head moderate, length 22.4–25.7% TL, scaleless. Snout gently convex, length 6.3–9.5% TL and 26.5–37.8% of head length, reaching slightly beyond upper lip. Orbit moderate, oval, length 3.0–5.6% TL, 12.0–22.3% of head length. Jaws moderate, reaching under front one-quarter or one-third of orbit. Mental crests low posteriorly, but anterior lobes developed and project; separated anteriorly by less than pupil diameter. Double row of

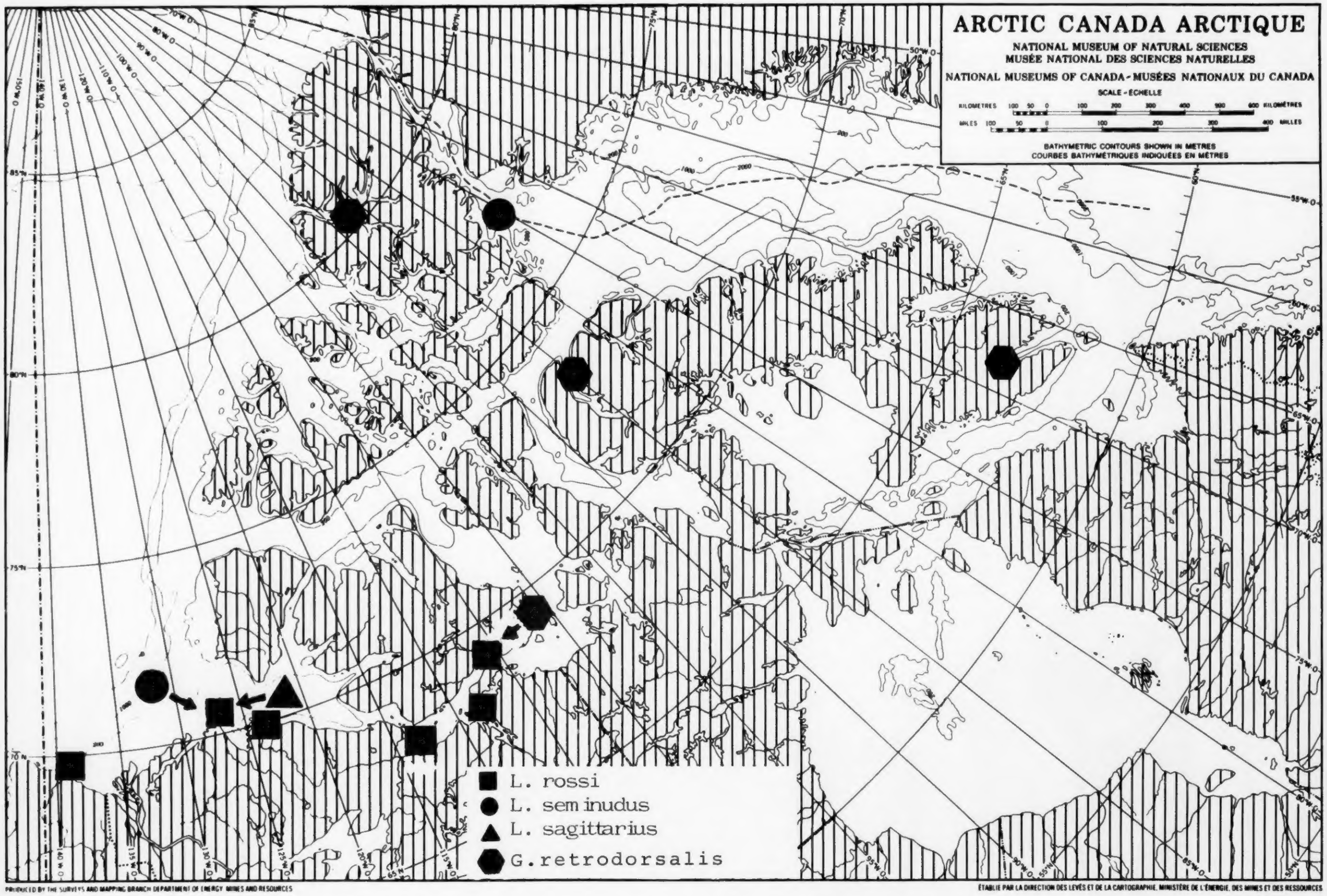
ARCTIC CANADA ARCTIQUE
NATIONAL MUSEUM OF NATURAL SCIENCES
MUSÉE NATIONAL DES SCIENCES NATURELLES
NATIONAL MUSEUMS OF CANADA - MUSÉES NATIONAUX DU CANADA
SCALE - ÉCHELLE
KILOMETRES 100 50 0 100 200 300 400 500 600 KILOMÈTRES
MILES 100 50 0 100 200 300 400 MILLES
BATHYMETRIC CONTOURS SHOWN IN METRES
COURBES BATHYMÉTRIQUES INDIQUÉES EN MÈTRES
■ L. rossi
● L. seminudus
▲ L. sagittarius
⬢ G. retrodorsalis
85°N
80°N
75°N
70°N
65°N
150°W
140°W
130°W
120°W
110°W
100°W
90°W
80°W
70°W
135°W
125°W
115°W
95°W
85°W
75°W
65°W
55°W
50°W
PRODUCED BY THE SURVEYS AND MAPPING BRANCH DEPARTMENT OF ENERGY MINES AND RESOURCES
ÉTABLIE PAR LA DIRECTION DES LEVÉS ET DE LA CARTOGRAPHIE, MINISTÈRE DE L'ÉNERGIE, DES MINES ET DES RESSOURCES

FIG. 2. Distribution of *Gymnelus retrodorsalis* and deep-water records of *Lycodes* in Arctic Canada.

teeth on premaxilla anteriorly, single posteriorly; same on lower jaw. Vomer with two to five (six) conical teeth. Palatines with single row of teeth. Sensory canal system on head opening through minute pores, skin forming dimples over chambers of system on mandible, sometimes over the suborbital canal. Opercular flap moderate, dorsal edge horizontal, not upwardly directed. Gill opening moderate, isthmus width moderate, comprising 5.1–6.5% TL.

Body — Body long and moderately slender, depth at anal origin 8.8–11.1% TL. Scales small, cycloid, isolated, covering most of body behind anal fin and extending forward in a wedge to about opposite middle of pectoral fin in specimens 65 mm TL or longer. Scales may be missing from tip of tail in specimens less than 100 mm TL or missing entirely (Jensen 1904) from specimens less than 50 mm. Scales absent from fins. Lateral line mediolateral, consisting of pale superficial neuromasts. Predorsal fin length 27.1–33.7% and preanal length 45.1–54.1% TL. Pectoral fin short, ovoid, margin curved, lower rays slightly exserted, length 11.8–15.2%, pelvics short, 2.4–4.4% TL, tips of pelvics not enlarged. A minute genital papilla behind anus in both sexes.

COLOR

Body and head a light grey–brown with light markings, scales pale. Upper head evenly pigmented except 2 (larger) out of 20 specimens have a light spot behind each eye and one small one has a dark stripe between the eye and the nostril. The light-colored nuchal bar sometimes breaks up into two to three spots. Underside of head light. Dorsal fin and back marked with six to nine, usually seven to eight, light bars which bend diagonally back on the fin. The light bars vary from half to equal the width of the intervening dark bars. In young the white bars fuse with the lightly colored region under the tail. The dark bars form darker borders next to the light bars (making the dorsal origin black). The abdomen tends to be darker than the rest of the underside of the body. Pectoral fin evenly grey colored. Peritoneum immaculate below, speckled above.

SEXUAL DIMORPHISM

None noted.

SIZE

The largest Canadian specimen known is 223 mm TL and weighed 63.6 g after preservation (NMC 77-976). Andriashev (1954) reports a specimen of 310 mm.

MATERIAL EXAMINED

Twenty specimens from Arctic Canada, 51-223 mm TL.

From Y.T.
NMC 60-476 (2), 11 km NE of Collinson Head, Herschel Island, at 91–120 m.

From N.W.T.
NMC 65–368 (3), Darnley Head off Cape Parry; NMC 67–302(1), Franklin Bay, at 70°3′54″N, 125°28′30″W; NMC 67-305 (2), Darnley Bay west of Cape Lyon, at 69°52′30″N, 123°05′30″W; NMC 67-307 (2), Franklin Bay, at 69°52′12″N, 125°49′W; NMC 77-975 (8), Franklin Bay east of Baillie Island, at 70°37′30″N, 127°20′W, in 236–248 m; NMC 77-976 (2), in Franklin Bay, at 335 m; NMC 77-1598(1), Dease Strait, at 69°10′06″N, 105°50′12″W; other specimens were identified, but not studied in detail, from Coronation Gulf and Bathurst Inlet (see distribution map (Fig. 2)).

Systematics

Andriashev (1954) discussed Knipovich's (1906) naming of four forms of *L. rossi* in the Barents Sea, stating that *L. rossi* is so close to *L. reticulatus* that it is hardly possible to differentiate *L. rossi* until the entire *rossi–reticulatus* complex is revised. However, he indicated that separation of those two species from the Barents Sea presents no great difficulties, where *L. reticulatus* has a deeper body, a longer pectoral with more rays, a more rounded opercular lobe, and different coloration. The color pattern of *L. rossi* is not unlike that of *L. raridens* (Andriashev 1954) but *L. raridens* has scales on the dorsal and anal fins and fewer teeth in the lower jaw. The color pattern also resembles that of *L. lavalei* Vladykov and Tremblay.

Distribution

The Dease Strait records extend the known range over 1300 km E from Herschel Island. The latter are over 3000 km from the next closest ones in Spitsbergen. Johsson (1960) reports the species from Iceland.

Biology

Known in Canada in depths between 47 and 335 m, salinities between 27.6 and 35.0‰, and temperatures between −1.5 and 1.1°C (but usually negative temperatures), on mud bottoms. Andriashev (1954, 1964) records it between 42 and 365 m (fry at 9 m), at temperatures below or close to 0°C and salinities usually not below 34‰.

About 50 specimens, ranging from 39 to 218 mm TL, have been captured in the Canadian Arctic. Weights of fresh caught specimens ranged from 0.3 g for a 39-mm, 8.9 g for a 110-mm, and 53.2 g for a 218-mm TL specimen. Andriashev (1954, 1964) reported remains of Amphipoda, Polychaeta, and *Propeamusium groenlandicus* in stomachs and that data on reproduction were not available. A 73-mm TL, 2.4-g Canadian Arctic specimen, caught August 28, contained remains of Polychaeta, Copepoda, Amphipoda, and three Cumacea (*Diastylis lucifera*) in its stomach.

Lycodes sagittarius
McAllister, 1976
Fig. 2 and 3

archer eelpout
lycode à arc

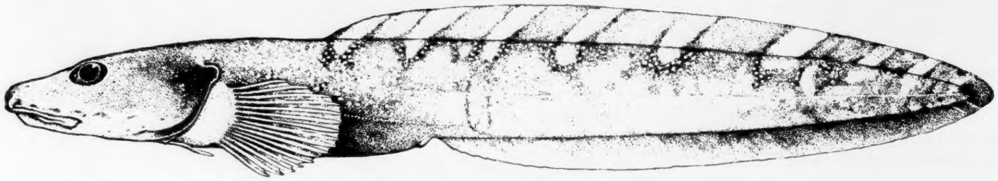

FIG. 3. *Lycodes sagittarius* (NMC 77-0976).

Synonymy

Original Description

Lycodes sagittarius McAllister, 1976: 9, fig. 1–3 (holotype: NMC 74-282, 278-mm male from arctic coast of Alaska about 81 km NNE of Brownlow Point, at 70°51′30″N, 145°17′W, in 357-m depth, August 9, 1972 (synon., diagn., descr., biol., relationships, key)).

Canadian Arctic Records

None.

Supplementary References

Lycodes squamiventer (*non* Jensen), Andriashev 1954: 297 (317 in translation), fig. 167, 169 (*ex parte*: two specimens from northern part of Kara Sea, USSR, at 595 m); 1973: 546 (*ex parte*: Kara Sea specimens).

Etymology — from the Latin *sagittarius*, an archer, in reference to the bow it carries in the anterior lateral line.

Diagnosis

Differs from other *Lycodes* in the Canadian Arctic by the lateral line bowed down anteriorly but returning to the midlateral position above the anal fin. Differs also in the black peritoneum, the 16–18 pectoral rays, the short preanal length, 38–43% TL, and in the scales covering most of the body and extending to the vertical fins of adults.

Description

Meristics

Range and sample size for the Canadian Arctic.

D + ½C 95–100 [15]
A + ½C 81–88 [10]
P 16–18 [26]
Br 6 [10]
GR 1–3 + 10–12 = 12–15 [10]
Abd. vert. 19–21 [26]
Caud. vert. 77–85 [24]
T. vert. 96–104 [24]
Pyl. caeca 2 [10]

Head — Head moderate, length 20.5–23.4% TL, scaleless. Profile gently convex. Snout length 6.1–7.5% TL and 28.9–34.2% of head length, extending beyond upper lip. Nostril reaches upper lip. Orbit large, transparent spectacle smaller, comprising 3.0–4.6% TL, diameter of pigmented portion larger, comprising 4.5–5.8% TL and 21.7–25.5% of head length. Jaws moderate, reaching below front third of orbit. Mental crests well developed, straight, in lateral view with tips bevelled or slightly projecting, separated by less than pupil diameter at tips. A single row of canine teeth posteriorly on the premaxilla and the lower jaw, both becoming double anteriorly. Vomer with two to five conical teeth; palatines with a single row of canine teeth. Head canal system not opening to the surface by pores in adults. Opercular flap usually horizontally directed. Gill opening long, isthmus width narrow, comprising 2.4–4.4% TL.

Body — Body long and moderately slender, depth at anal origin 8.1–9.9% TL. Scales small, cycloid, isolated, covering most of body except for the anterior nape, base of pectoral fins, and pelvic region in Canadian specimens (but larger Alaskan specimens were better scaled in these regions). Scales extend two-thirds of the height of the dorsal fins in specimens 135 mm TL, lesser heights in smaller specimens. About 20–27 horizontal rows of scales across body. Lateral line descends behind head to ventrolateral position above level of the anus, where it ascends to midlateral position and continues to end of tail. Predorsal length 27.7–31.0%, and preanal length 40.7–43.3% TL. Pelvics slender, long, length about equal to transparent orbit and comprising 3.0–5.2% TL; tips slightly swollen. Pectorals short, margin ovoid, lower rays slightly exserted, length 10.3–12.9% TL. A minute genital papilla behind anus in both sexes.

Color

Body and head a grey–brown. Underside of head lighter, operculum often duskier. Abdomen darker than rest of body. A few specimens monotone but most Canadian specimens have seven to nine light bars on the dorsal fin, where they angle posteriorly, and the upper back. In adults the bars tend to be restricted to the back and to be narrower while in smaller specimens they tend to extend below the midline and are broader. The width of the light bars is about one-third to one-half the dark interspaces. In some small specimens the dark bars become black on the dorsal fin. The color of Canadian specimens contrasts with that of Alaskan and Soviet material which was dark brown without light bars in most specimens. The scales and neuromasts are lighter than the background. Peritoneum varies from solid black to sooty.

SEXUAL DIMORPHISM

None noted.

SIZE

The largest Canadian specimen is 180 mm TL and preserved weighs 18.6 g. The largest known specimen is the holotype from Alaska which measures 278 mm TL and weighs 71.4 g.

MATERIAL EXAMINED

Twenty-six specimens of which 10 were examined in detail and which measured 98.2–180 mm in TL.

From N.W.T.
NMC 77-976 (26), Franklin Bay, 35 km E of Baillie Island, Cape Bathurst, in 335 m. Also examined were the holotype and nine paratypes.

Systematics

The N.W.T. specimens agree in most respects with the type series. They do differ in possessing light bars and in total vertebral counts. Twenty-four N.W.T. specimens have 96–104 vertebrae and (16)17–18 pectoral rays while nine paratypes from Alaska and the Kara Sea have 104–109 vertebrae and 16–17(18) pectoral rays. It is difficult to compare morphometry as only one specimen in each series is about the same size. It is tempting to conclude that the N.W.T. specimens represent a new species, especially since the distant Kara and Alaskan specimens have the same counts and color. But it is unlikely that there are two species of long-tailed eelpouts with bowed lateral lines and elongate pelvic fins in the deeper waters of the Beaufort Sea. Hence we take the conservative view and interpret the differences as population variation until more specimens become available.

This species may be related to some Pacific species with bowed lateral lines as suggested by McAllister (1976) or to deep, dark, high-count species such as *L. concolor* or *L. albolineatus*.

Distribution

The Franklin Bay record extends the known range eastward over 750 km from the offing of Brownlow Point, Arctic Alaska, and provides the first record for Canada. The species is also known east of Graham Bell Island, USSR, in the Kara Sea. The paucity of records is doubtless due to its relatively deep habitat, 335–600 m, and the rarity of collections from deep water in the Arctic. Its absence from collections made in waters of sufficient depth in Davis Strait and Baffin Bay suggests that it is lacking there and that the species may be confined to the central Arctic basin.

Biology

The Franklin Bay specimens were caught at a depth of 335 m, temperature of 0.30°C, salinity of 34‰, on a mud and clay bottom. In Arctic Alaska they were caught between 357 and 600 m and in Kara Sea, USSR, at 595 m, −0.91°C, 34.96‰, and an oxygen saturation of 88%, on a brown mud bottom.

The few available data suggest later summer or early fall spawning. Stomachs of Alaskan specimens contained annelids, bivalves, gastropods, and crustaceans. The numerous vomerine teeth, round-tipped in large specimens, may be an adaptation to a durophagus molluscan diet.

Lycodes frigidus Collett, 1878 — cold water eelpout / lycode glaciale

Synonymy

ORIGINAL DESCRIPTION

Lycodes frigidus Collett, 1878: 45 (15 syntypes deposited at ZMUO, ZMUC, BMNH, MNHN, and USNM, all from Norwegian Sea and off Spitsbergen, Norwegian North Atlantic Expedition, 1876–78, 475–2438 m).

CANADIAN ARCTIC RECORDS

None.

SUPPLEMENTARY REFERENCES

Lycodes frigidus, Collett 1880: 96–103, pl. III, fig. 23, 24 (descr., distr., depth); Jensen 1904: 22–25, pl. V, fig. 1a, b (synon., descr., depth); Andriashev 1954: 302–305 (324–326 in translation), fig. 172 (synon., descr., distr., life hist.); 1964: 374–375 (descr., distr.).

Etymology — From the Latin *frigidus* for cold or cool waters in which this species is found; it is usually collected at temperatures below 0°C.

Diagnosis

Differs from other *Lycodes* in the area by the very small scales, about 42–48 in irregular, longitudinal series on body at vertical through anus, body color monotone, greyish or brownish, lateral line with complete, ventrolateral branch only, vertebrae 103–107, anal fin rays 85–90, including half the caudal rays.

Description

MERISTICS

Represents only the Chukchi Rise specimen.

D + $\frac{1}{2}$C 101
A + $\frac{1}{2}$C 86
C 10
P 20
Br 6
GR 4 + 11 = 15
Abd. vert. 23
Caud. vert. 81
T. vert. 104
Pyl. caeca 2

Head — Head moderate, length 24.5% TL, scaleless. Snout gently convex, tip evenly rounded, length 6.4% TL and 25.9% of head length, slightly overhanging upper jaw. Orbit large, ovoid, length 7.9% TL, 32.2% of head length. Jaws moderate, reaching to half the orbit diameter. Mental crests well developed, no anterior flap, separated anteriorly by about one-half pupil diameter. Single row of moderate, sharp teeth in premaxilla with two teeth behind row at anterior tip in our young specimen, adults with more but smaller teeth behind outer row; lower jaw with double row of teeth on anterior half, single row posteriorly. Vomer with five relatively large, conical teeth. Each palatine bone with single row of 12 large teeth. Sensory canal system on head opening through minute pores, many not developed in our young fish, cavities leading to foramina in bones beneath skin cavernous, reminiscent of the condition in *Bothrocara*. Gill opening well developed, extending forward dorsally creating moderate, posteriorly directed flap; ventrally extending slightly in front of pelvic fin base; isthmus width small, comprising 4.3% TL.

Body — Body long and slender, depth at anal origin 10.2% TL. Scales had just begun to develop slightly posterior to the anus in our young specimen, restricted to ventral portion and extending only a short distance down tail; scales in adults minute, imbedded, not overlapping one another, covering tail and body, on abdomen to pelvic base and on bases of unpaired fins, but not on pectoral base or head. Lateral line ventrolateral, complete. Predorsal length 29.4% and preanal length 46.0% TL. Pectoral fin moderate, ovoid, upper rays longest, comprising 14.2% TL. Pelvic fins short, 3.3% TL with three rays each.

COLOR

Collett (1880) reports fresh color as uniformly dark reddish-grey or brownish-grey, with young individuals lighter than adults. Abdomen and branchiostegal membrane reported to be bluish-black (Andriashev 1954). Under surface of head lighter than upper surface and body.

SEXUAL DIMORPHISM

Not noted, never reported.

SIZE

The Chukchi Rise specimen is a juvenile female 143 mm TL. Nizovtsev et al. (1976) reported the maximum known length of 690 mm TL.

MATERIAL EXAMINED

One specimen from off the Chukchi Rise, 143 mm TL, and five specimens from the Arctic Atlantic, all syntypes, 270–375 mm TL.

From Chukchi Rise
MCZ 40707, 77°37.7′N, 172°43.0′W, 2215 m, November 30, 1959.

From Arctic Atlantic
ZMUO 4517, 4518, 71°59′N, 11°40′E, 2030 m, July 14, 1878; ZMUO 4519, 75°12′N, 03°02′E, 2195 m, July 19, 1878; ZMUO 4520, 77°59′N, 05°10′E, 2438 m, August 10, 1878; ZMUC 24, 80°03′N, 08°28′E, 475 m, August 14, 1878.

Systematics

Andriashev (1954, 1964) summarized the confusion in the literature on *L. frigidus*. Two closely related species, *L. atlanticus* Jensen, known along temperate Atlantic slope from off Ireland to Florida, and *L. agulhensis*, from off southern Africa, have been wrongly ascribed to *L. frigidus*, which appears to be confined to the Arctic Ocean.

Distribution

The Chukchi Rise specimen extends the known range 6500 km E from off Bol'shevik Island, Laptev Sea (Andriashev 1954). A bottom photograph taken on the Chukchi Rise, at 78°28′N, 171°10′W, in 2653 m (about 430 km NE of the capture locality of our specimen), published by Menzies et al. (1973, fig. 6–20a), is probably *L. frigidus* also. The species is well known from the depths of the Greenland Sea north of Iceland, the Norwegian Sea north of the Faeroe-Iceland Ridge, and the Barents Sea.

Lycodes eudipleurostictus Jensen, 1902 — doubleline eelpout
lycode à deux lignes (f.)

Synonymy

ORIGINAL DESCRIPTION

Lycodes eudipleurostictus Jensen, 1902: 206 (syntypes: ZMUC 138, north of Faeroe Islands, Danish Ingolf Exped., St. 138, at 63°26′N, 7°56′W, 890 m; ZMUC 7, Umanak Fjord, west Greenland).

CANADIAN ARCTIC RECORDS

None.

SUPPLEMENTARY REFERENCES

Lycodes eudipleurostictus, Jensen 1904: 32−37, pl. III, fig. 1a, b (synon., descr., taxon., distr., depth); 1952: 11−14 (synon., descr., dist., depth); Andriashev 1954: 320−322 (298−299 in translation), fig. 168 (synon., descr., distr., life hist.).

Etymology — *eudipleurostictus*, from the Greek *eu* for true, *di* for two, *pleur* for side, and *stictus* for dotted in reference to the two lateral lines on the sides composed of lateral line organs, each forming a light-colored spot.

Diagnosis

Differs from other *Lycodes* in the Canadian and Alaskan Arctic by the complete mediolateral and ventrolateral branches of the lateralis system and the shallowly forked pectoral fin, the uniform dark brown coloration with 9 or 10 simple, narrow, yellowish-white stripes running from dorsal fin to midbody except on tail where they extend onto anal fin. Preanal length short, 39.3−43.9% TL; scales cover body and tail, including abdomen, extend onto dorsal and anal fins (in adults) but are absent on nape, head, pectoral fin base, and axil.

Description

MERISTICS

Range and sample size for Beaufort Sea.

D + ½C about 107−112 [2]
A + ½C 92−96 [2]
C 8−9 [2]
P 20 [2]
Br 6 [2]
GR 4 + 11−12 = 15−16 [2]
Abd. vert. 12−23 [2]
Caud. vert. 86−91 [2]
T. vert. 109−112 [2]
Pyl. caeca 2 [2]

Head — Head moderate, length 21.5−24.2% TL, scaleless. Snout gently convex, steep anteriorly, length 5.5−6.4% TL and 26.0−26.4% of head length. Snout tip and upper jaw slightly overhanding lower jaw. Nostril short and not reaching upper lip. Orbit large, ovoid, length 6.7−6.9% TL. Jaws moderate, reaching to below front one-third to one-half of orbit. Mental crests low, narrowly separated anteriorly. Lower lip skin fold well developed. A single row of canine teeth on premaxilla, our specimens with one to four smaller teeth behind canines at symphysis; one row on lower jaw posteriorly, triple row at dentary symphysis. Vomer with four to six teeth, conical and blunt. Palatines with a single row of 11−12 relatively large, stout teeth. Head canal system opening through minute pores, skin forming low dimples over chambers on lower jaw and over suborbital canal. In our adults, anterior pores appear closed or openings greatly constricted. Opercular flap moderate, dorsal edge horizontal. Gill opening long, reaching to below ventral base of pectoral fin, isthmus moderate, comprising 5.2−5.5% TL.

Body — Body long and moderately slender, tail more laterally compressed. Depth at anal origin 11.1−11.9% TL. Scales relatively large for a *Lycodes*, cycloid, diminishing in size posteriorly. Scales cover almost entire body, the abdomen to just behind pelvic fin base and onto dorsal and anal fins; scales absent on head, nape, pectoral fin and its base and axil. Lateral line with complete ventrolateral and mediolateral branches, bifurcation of branches about one-quarter pectoral fin length posterior to pectoral base. Anteriormost section of ventrolateral branch very steep, nearly vertical. Predorsal length 23.8−26.7% and preanal length 39.3−43.9% TL. Pectoral fin moderate, lowermost six rays with thickened tips, more widely exserted than upper rays and slightly longer than uppers, producing a notch in middle of fin. Notch not as deep as in *L. diapterus* or *L. taranetzi*, less evident when fin expanded. Pectoral fin length 12.5−12.9% and pelvics 2.7−2.9% TL. Minute genital papilla in both specimens.

COLOR

Our specimens, in alcohol, are somewhat faded, although the typical, simple, light stripes along the dorsum are visible. Body and tail uniformly dark brown, head lighter, nuchal bar visible in both. All fins brown, dorsal with 9 or 10 light bands extending onto body, posteriormost 5 run onto anal fin. Jensen (1904, 1952) gives the range of these bands as 5−13. Bands whitish-yellow in life (Jensen 1904). Lining of mouth brown, gill chamber and peritoneum black.

SEXUAL DIMORPHISM

Both our specimens were males.

SIZES

The largest Beaufort Sea specimen measures 346 mm TL. Jensen (1952) reports specimens to 445 mm from Greenland.

Material Examined

Two specimens from the Beaufort Sea, Arctic Alaska, 315 and 346 mm TL and one from Greenland.

From Alaska
NMC 74-275 (2), north of Kaktovik, at 70°43.1′N, 143°42.8′W, in 464 m.

From Greenland
NMC 80-415 (1), southwest Greenland, at 66°11′N, 56°43′W, in 25−260 m, 4.31°C.

Systematics

Jensen (1904) separated this species from *L. esmarki*, found in the same habitats, by its complete mediolateral and ventrolateral lateral lines, indented pectoral fin, the different coloration, and the presence of two small pyloric caeca;

he also considered the two species distinct in that *L. eudipleurostictus* had fewer vertebrae and consequently fewer unpaired fin rays. As considerable geographic variation in vertebral counts has been found in other eelpouts (Jensen 1902; Schmidt 1917a, b; Peden 1979; M. E. Anderson, Virginia Institute of Marine Science, Gloucester Point, VA 23062, USA, unpublished), we feel Jensen's latter claim requires verification from more specimens than he examined.

Distribution

The Alaskan specimens extend the range southwestward over 2400 km from Smith Sound, northwest Greenland (Jensen 1952). The species is also known from the Kara Sea (north of Severnaya Zemlya), the Barents Sea, off Spitsbergen, the coast of Norway, Wyville-Thomson Ridge, Iceland, northeast Greenland, and dozens of localities off western Greenland (Jensen 1904, 1952; Andriashev 1954).

Lycodes squamiventer scalebelly eelpout
Jensen, 1904

Synonymy

ORIGINAL DESCRIPTION

Lycodes pallidus squamiventer Jensen, 1904: 39, pl. IV, fig. 2a, b (southern part of Norwegian Sea between Norway and Greenland; syntypes: *Ingolf* Sts. 101, 104, 105, 141 and *Michael Sars* St. 36, 1015−1808 m); Nielsen, 1974: 77 (list of syntypes).

CANADIAN ARCTIC RECORDS

None.

SUPPLEMENTARY REFERENCES

Collett 1909: 135 (fecundity); Andriashev 1954: 295 (317 in translation) (in part, excluding Kara Sea records and excluding fig. 167, 169); 1973: 546 (synon., in part).

Etymology — *squamiventer*, from Latin *squama* for scale and *venter* for belly, in reference to the characteristic scales on the abdomen.

Diagnosis

Differs from other *Lycodes* in the double lateral line, the about 17 pectoral and 87 anal (including half the caudal) rays, and the black peritoneum. Scales cover the lower two-thirds of the dorsal and anal fins as well as the belly.

Description

MERISTICS

Based on single Alaskan specimen with counts for Norwegian Sea specimens in parentheses.

D + $\frac{1}{2}$C 99 (94−97)
A + $\frac{1}{2}$C 87 (81−86)
P 17 (17−21)
Br 6
GR 3 + 12 = 15 (14)
Abd. vert. 20 (19−21)
Caud. vert. 84 (77−82)
T. vert. 104 (97−101)
Pyl. caeca 2 (2)

Head — Head moderate, 22.3% TL, scaleless. Snout slightly convex, length 7.9% TL and 35.5% of head length, not overhanging upper lip. Orbit moderate, oval, length 3.2% TL and 14.3% of head length. Jaws moderate, reaching below midorbit. Mental crests low, anterior part higher and ends bevelled; separated anteriorly by less than pupil diameter. Double row of teeth anteriorly and single posteriorly on premaxilla and lower jaw. Vomer with two, palatine with single row of nine conical teeth. Opercular flap moderate, upwardly directed. Gill opening narrow, isthmus width narrow, comprising 3.5% TL.

Body — Body long and moderately slender, depth at anal origin 9.8% TL. Scales small, cycloid, dense, covering all of body except for naked area behind occiput. Scales on lower two-thirds of dorsal and anal fins. Two lateral lines, first descending from head to anal origin and continuing above anal base to opposite midpoint of anal fin, second mediolateral on urosome and continuing anteriorly almost to above anal origin. Predorsal length 30.8% and preanal length 41.4% TL. Pectoral short, ovoid, length 11.3% TL. A minute genital papilla behind anus in female specimen.

COLOR

Head and body a monotone dark brown with no visible markings except top of head somewhat lighter. Peritoneum solid black.

SEXUAL DIMORPHISM

None noted.

SIZE

Only Alaskan specimen is 197 mm TL, but reported to 260 mm by Jensen (1904).

MATERIAL EXAMINED

One specimen from Arctic Alaska, 197 mm TL, and four syntypes from between Greenland and Norway, 144−242 mm TL.

From Arctic Alaska
NMC 74-282A(1), 85 km NNE of Brownlow Point, at 70°51′30″N, 145°17′W, at 357 m.

From between Greenland and Norway
ZMUC 235−237, southern Norwegian Sea; ZIL 4718(1),

Ingolf St. 105, at 65°34′N, 7°31′W, in 1435 m, X ray only.

Systematics

There are some meristic differences between the Alaskan specimen and those from the Norwegian Sea. The Alaskan specimen has 99 dorsal and 87 anal rays versus 94−97 and 81−83 in the Norwegian Sea specimens. The Alaskan specimen has a count of 104 versus 97−101 total vertebrae. Given the small samples studied this does not seem worthy of taxonomic recognition. Alaskan and Norwegian Sea samples did not differ significantly morphometrically.

Distribution

The Alaskan specimen extends the known range from the Norwegian Sea near Iceland more than 4000 km W.

Lycodes seminudus
Reinhardt, 1837
Fig. 2 and 4

longear eelpout
lycode à oreilles (f.)

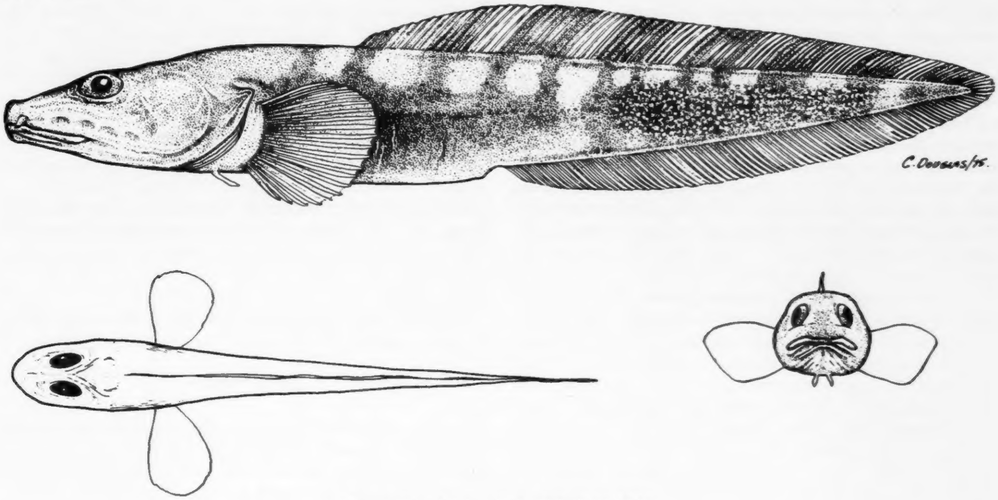

FIG. 4. *Lycodes seminudus* (NMC 71-0768).

Synonymy

ORIGINAL DESCRIPTION

Lycodes seminudus Reinhardt, 1837: 117 (Omenak-Umanak Fjord in west Greenland, at 71°N; from stomach of an unknown fish; published again in 1838); Nielsen, 1974: 78 (holotype: ZMUC No. 9, Umanak, Greenland).

CANADIAN ARCTIC RECORDS

Lycodes seminudus, Jensen 1952: 21 (Jones Sound, N.W.T., at 75°54′N, 81°01′W, four specimens in trawl at 610 m and −0.59°C); Legendre et al. 1975: 9 (new French and English vernaculars, no locality given, based on NMC 71-768 from Baffin Bay, N.W.T.).

SUPPLEMENTARY REFERENCES

Lycodes seminudus, Jensen 1904: 71, 98, pl. IX, fig. 1a, b, c, d, e, pl. X, fig. 1a, b, text fig. 11−14 (synon., descr., taxon., distr., depth, temp.); 1952: 18 (synon., distr., depth, color, eggs); Andriashev 1954: 281 (302 in translation), fig. 152, 153 (synon., descr., distr., life hist.).

Etymology — *seminudus*, from the Latin half-naked, in reference to the absence of scales from the front part of the body.

Diagnosis

Differs from other *Lycodes* in the region by the combination of a mediolateral lateral line and dusky peritoneum. Also characteristic are its numerous, 19−20, pectoral rays, scales restricted to behind the end of the pectoral fin, the shortness of the pectoral fin, 9−12% TL, and the 74−76 rays in the anal fin including half the caudal rays.

Description

MERISTICS

Range and sample size for the Canadian Arctic.

D + $\frac{1}{2}$C 93−97 [7]
A + $\frac{1}{2}$C 74−78 [7]
P 19−20 [7]
Br 6 [6]
GR 2−3 + 10−13 = 13−15 [7]
Abd. vert. 23−26 [7]
Caud. vert. 71−74 [7]
T. vert. 96−98 [7]
Pyl. caeca 2 [7]

Head — Head moderate, length 24.5−26.5% TL, scaleless. Snout flat or dished out, length 6.5−8.7% TL and 25.6−33.3% of head length, reaching slightly beyond upper lip. Nostril reaching upper lip. Orbit large, oval, length 4.8−5.6% TL and 18.4−23.4% of head length. Jaws moderate, reaching below front third of orbit. Mental crests low, narrowly separated anteriorly. A single row of canine teeth on premaxilla (occasionally 2−4 small teeth behind this row of symphysis), one row on lower jaw becoming double anteriorly. Vomer with three to seven conical teeth; palatines with a single row of canine teeth. Head canal system not opening to the surface by pores in adult, the suborbital canal marked by depressions in the skin. Opercular flap longer and more dorsally directed than in other *Lycodes*. Gill opening long, reaching below base of pectoral fin, isthmus narrow comprising 3.5−5.9% TL.

Body — Body long and moderately slender, depth at anal origin 8.3−11.3% TL. Scales small, cycloid,isolated, covering most of body behind anal fin in specimens over 135 mm TL, scales forming a wedge anteriorly which reaches to or just in front of anus, rarely it reaches to the middle of the pectoral fin in large specimens. Approximately 20 horizontal rows of scales across body. Scales absent from fins. Lateral line mediolateral. Predorsal length 30.3−32.9% and preanal length 46.8−50.1% TL. Pelvics small, slender, tips not enlarged, length less than orbit diameter and comprising 3.3−4.2% TL. Pectorals short, margin rounded, lower rays slightly exserted, length 9.4−12.1% TL. Pyloric caeca, short, two. A minute genital papilla behind anus in both sexes.

COLOR

Body and head a light grey−brown. Snout, occiput, and cheek darker than area below eye. Underside of head light. Abdomen darker than rest of body. Dorsal and upper back usually marked with four to eight light bars (one of seven Canadian and three of three Alaskan specimens without stripes). Bars pale, wide as pupil, diagonal on dorsal fin and vertical on back where they reach down one-third to one-half of the way to the lateral line before they fade out. Pigment next to the white bars is more intense, giving them a darker border. Jensen (1904) mentioned two color varieties, one uniformly colored, the other with crossbands. Scales and neuromasts are lighter than the background. Pectoral fin dark with posterior border light-edged. The peritoneum is sooty, one-half of its surface or more being covered with black speckles in Canadian material (two Alaskan specimens have solid black peritoneum but the third and largest has only lightly speckled peritoneum).

SEXUAL DIMORPHISM

The five largest specimens known are males. The head tends to be larger in males than in females, 27.0−28.5% as opposed to 25.0−28.0%, and the preanal distance tends to be longer, 46.1−50.6% as opposed to 44.6−48.3% TL (Jensen 1904).

SIZE

The largest Canadian specimen is 193 mm TL and preserved weighs 27.1 g, and the largest Alaskan specimen is 242 mm TL and preserved weighs 98.2 g. Jensen (1904) mentioned a male specimen of 497 mm taken in west Greenland and (1952) one of 517 mm TL from Upernavik, west Greenland.

MATERIAL EXAMINED

Six specimens from Arctic Canada, 101−193 mm TL, and three specimens from Alaska, 142−292 mm TL.

From N.W.T.
NMC 71−768 (1), Baffin Bay off southeast Ellesmere Island, at 77°12′42″N, 75°31′24″W, in 570−750 m; NMC 77-976 (5), Franklin Bay 35 km E of Baillie Island, in 335 m.

From Alaska
NMC 74-282A (3), about 81 km NNE of Brownlow Point, Beaufort Sea, in 357 m.

Systematics

Jensen (1952) described a new species, *L. nigricans*, from Greenland specimens which resembled *L. seminudus* except that in *L. nigricans* the adults are dark without markings and the body scale covering reaches a level even with the tip of the pectoral fin and continues as a single row of scales above the gill cover to the head; only very rarely do the scales reach forward only to near the tip of the pectoral in *L. seminudus*. Andriashev (1954) reported similar dark specimens from the Kara Sea and believed there were no grounds for excluding them or Jensen's specimens from *L. seminudus*. Andriashev (1973) included *L. nigricans* in the synonymy of *L. seminudus*. However, *L. nigricans* differs in the presence of a dorsal branch of the lateral line system, coloration, and longer, sharper teeth. So we regard them as distinct pending further study.

Two Alaskan specimens 142 and 158 mm TL have the scales continuing forward to a point halfway between the anus and the pectoral fin, while a third specimen with light markings, 292 mm TL, has the scales extending forward on the body to opposite the midpoint of the pectoral fin.

Distribution

The Franklin Bay records extend the known range southwestward from Jones Sound, N.W.T., more than 1400 km, the Alaskan record (NMC 74-282A) more than 1900 km SW. Both of these records are about 3000 km from the nearest Soviet record in the northern Kara Sea. As Andriashev (1954) suggests, the species will probably be found in eastern Soviet northern seas when collections are made at the edge of the continental shelf. Jonsson (1969) reports the species from Iceland.

Biology

In Canada specimens have been caught between 335 and 610 m, at temperatures of −0.59 to +0.30°C, salinities of 34‰, on mud or mud clay bottoms. Andriashev (1954) recorded them between 130 and 1100 m, almost exclusively below 0°C, at salinities exceeding 34.5‰, and mainly on mud bottoms.

Andriashev (1954) reported bottom Amphipoda, Decapoda (*Sabinea*), Isopoda, and Polychaeta in stomachs. He had no data on reproduction.

Subfamily GYMNELINAE

Genus *Gymnelus* Reinhardt, 1834

Gymnelus Reinhardt, 1834: 4 (type: *Ophidium viride* Fabricius, by monotypy; gender masculine)
Gymnelis Reinhardt, 1837: 34, 49 (emended after *Gymnelus* Reinhardt, 1834; Reinhardt 1838: 116, 131)
Cepolophis Kaup, 1856: 96 (type: *Ophidium viride* Fabricius, by original designation)
Gymnelichthys Fischer, 1885; 60 (type: *Gymnelichthys antarcticus* Fischer, by monotypy)
Nemalycodes Herzenstein, 1896: 14 (type: *Nemalycodes grigorjewi* Herzenstein, by monotypy)

Etymology — *Gymnelus* from the Greek words for naked and eel in reference to the scaleless skin and eel-shaped body of this genus. Although the author amended his first spelling *Gymnelus* to *Gymnelis* in 1837 and many authors have followed the latter spelling, the original spelling, *Gymnelus*, is the correct one.

Body very elongate, scaleless; lateral line mediolateral; gill openings small, not reaching ventral edge of pectoral fin base; lips of upper and lower jaws about even; vomer and palatines toothed; infralabial lobes on lower jaw separated by narrow space; submental crests absent; cephalic lateralis pores well developed; palatal and mandibular membranes well developed; dorsal fin without short spinelike rays in posterior third of fin; pelvic fins absent; two short pyloric caeca; vertebrae 85−105; vertebral centra asymmetrical (with constriction closer to anterior end).

At least four species in Arctic Ocean and Bering Sea: *G. viridus*, *G. retrodorsalis*, and *G. hemifasciatus* in the Arctic, and the latter and *G. bilabrus* in the Bering Sea. According to Andriashev (1954) *Gymnelus* is closely related to *Commandorella*, *Gymnelopsis*, and *Derjugina* from the Bering Sea and the Sea of Okhotsk. M. E. Anderson is currently revising these four groups.

Gymnelus retrodorsalis
Le Danois, 1913
Fig. 2 and 5

aurora pout
unerak aurora

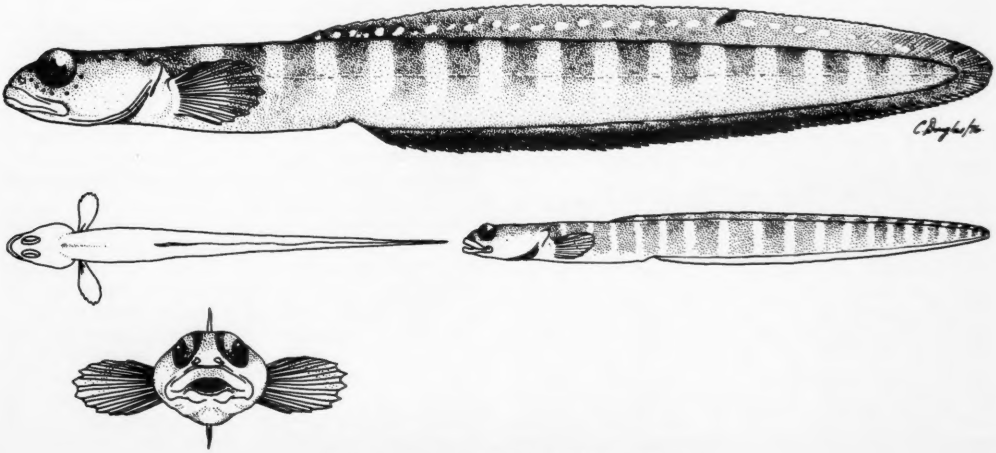

FIG. 5. *Gymnelus retrodorsalis* (NMC 75-1953).

Synonymy

ORIGINAL DESCRIPTION

Gymnelis retrodorsalis Le Danois, 1913: 258−259 (to south of Jan Mayen, at 80°33′30″N, 8°37′W, in 140 m by ship *Pourquoi-Pas*?)

CANADIAN ARCTIC RECORDS

None.

SUPPLEMENTARY REFERENCES

Gymnelis retrodorsalis, Le Danois 1914: 16, 58, fig. 5, 22, pl. fig. 1−2 (descr., distr.); Andriashev 1954: 262 (283 in translation), fig. 136−138 (synon., descr., X ray, taxon., distr., life hist.).

Etymology — *retrodorsalis*, from the Latin *retro* for behind and *dorsum* for back in reference to the posterior position of the origin of the dorsal fin.

Diagnosis

The genus differs from *Lycodes* in lacking pelvic fins. Differs from *G. viridus* and *G. hemifasciatus* in the shorter dorsal fin which begins behind the tip of the pectoral fin instead of over it; 3−14 dorsal pterygiophores present at front of fin without rays versus 0−2 as in *G. viridis* and *G. hemifasciatus*; predorsal distance 25−31% versus 14−20% TL.

Description

MERISTICS

Range and sample size for the Canadian Arctic.

D + ½C 87−92 [11]
A + ½C 77−84 [11]
C 9−13 [5]
P 10−12 [9]
Br 6 [4]
GR 2−4 9−12 13−15 [6]
Abd. vert. 20−22 [16]
Caud. vert. 72−80 [16]
T. vert. 92−100 [16]
Pyl. caeca 2 [2]
Rayless dorsal pterygiophores 6−12

Head — Length short, 15.6−16.8%, width 6.9%−9.7% TL, width less than head depth. Profile gently convex. Snout length 3.1−3.8% TL, 19.4−23.2% of head length. Nostril reaches to or almost to upper lip. Orbit large, diameter 3.9−4.9% TL, 24.8−33.1% of head length. Gape of jaws when closed, at angle of 10−15°. Jaws large, upper jaw reaches from below midpupil to between pupil and posterior margin of orbit, length 5.8−7.8% TL, lower jaw equal to or slightly shorter than upper. Mental crests absent. Single row of canine teeth posteriorly, becoming double anteriorly with 16−32 on the premaxillary and 17−33 on the mandible. Vomer with 6−9 conical teeth, palatines with a single row of 8−14 canine teeth. Three patches of teeth on upper pharyngeals, one on lower pharyngeals. Preoperculomandibular canal with seven to eight pores, suborbital pores six to eight, usually six, postorbital pores four, occipital pores two, supraorbital pores two, and no interorbital pore. Gill rakers triangular, short, length less than one-half of pipil diameter. Gill opening small, length about equal to orbit diameter, extending from above pectoral base to opposite middle of pectoral fin base.

Body — Body very slender, subcylindrical, depth at anal origin 6.3−8.6% TL, width of body at anal origin about one-half to two-thirds of depth. Lateral line obscure, mediolateral, extending to tail tip. Dorsal origin usually about an orbit diameter behind tip of pectoral, but varying from tip of pectoral to one and one-third orbit diameters behind tip of pectoral. Predorsal length 25.0−30.8% and preanal length 33.8−37.7% TL. Pelvic fins absent. Pectoral fin small, shape ovoid, rays slightly exserted, length 7.4−9.1% TL, basal width 23.2−37.0% (39%) of its length. Pyloric caeca two, one on right side slightly longer. A minute genital papilla in both sexes. Vent about a pupil diameter in front of anal origin.

COLOR

Preserved specimens. Fifteen of the 17 specimens have 14−17 dark vertical bars along the side of the body, separated by pale areas narrower than or equal to the width; one specimen has a series of A-shaped dark markings along the side, and one apparently lacks markings. Male specimens have dark anal fins, females pale ones. Three specimens have a single dark ocellus surrounded by a light ring in the middle of the dorsal fin, two have two ocelli, one has six ocelli, the remainder lacks them. The underside of the head, and pectoral and caudal fins are light. The abdomen is pale or lightly pigmented. One specimen has a dark horizontal line on the head at the level of the eye, otherwise there are no markings on the head. Peritoneum, buccal and branchial cavities are pale.

The type from south of Jan Mayen has 20 brownish bars on the sides and a white-bordered black ocellus between the 16th and 17th dorsal rays, and a gray anal fin marbled with brown. Andriashev (1954) reports that the color varies and can hardly be used as a diagnostic feature.

The following notes are based on color slides and observations of a freshly caught specimen from Frobisher Bay kept alive in an aquarium (NMC 70-280). Background color of body and head a translucent cream color with bars crossing dorsal fin diagonally and body vertically from back to ventral surface. Six ovoid ocelli on dorsal fin slant backwards, each about the size of the orbit, with a black center encircled by an intense white band. Pectoral whitish, rest of fins transparent, aside from marks on dorsal fin. Iris silvery. Another specimen is similar except it has a single ocellus in the dorsal fin, and the abdominal and opercular regions are a reddish-brown (NMC 70-278, Frobisher Bay).

SEXUAL DIMORPHISM

Two males had dark anal fins, the three females had light ones. However, the anal fin turned from dark to light in ~1 min in one live specimen. Both males had a single dark ocellus in the middle of the dorsal fin, one female had two ocelli, the other two females none. Andriashev (1954) indicates that the males tend to have wider heads, 8.9−10.2%, than the females, 7.2−8.6%, and longer upper jaws, 6.6−8.5%, than females, 5.7−6.5% TL. Our five specimens tend to support this and additionally suggest that the head length is relatively longer in males, 16.2−16.8%, than in females, 15.6−16.0% TL.

SIZE

The largest Canadian specimen is 125 mm TL and weighs 4.6 g preserved. Andriashev (1954) reports specimens from the Barents and Kara seas up to 140 mm TL.

MATERIAL EXAMINED

Seventeen specimens of which six were measured in detail and which ranged from 90 to 125 mm TL.

From N.W.T.

NMC 70-278 (1), Frobisher Bay, 8 km SSE of head of bay, southeast Baffin Island, in 68 m; NMC 77-1501B (1), Frobisher Bay, ~3 km E of eastern tip of Laird Peninsula, in 68 m; NMC 77-1092 (1), Nanson Sound, 1.5 km W of Butter Porridge Point, Schei Peninsula, Axel Heiberg Island, in 8 m; NMC 75-1953 (14), Strathcona Sound, Borden Peninsula, northeast Baffin Island, in 250−300 m.

Systematics

According to Andriashev (1954) "The final determination of the species status of *G. retrodorsalis* is impossible without a description of specimens from more westerly regions (Greenland and adjacent islands of the Canadian Archipelago . . . ." The authors were tempted, as was perhaps Andriashev, to consider that *G. retrodorsalis* might really consist of aberrant specimens of *G. viridis* which lost the first few dorsal rays. Jensen (1938) went so far as to synonymize the two species. But the two species defined by *G. viridis* having 0−1, rarely 2, dorsal pterygiophores without rays and *G. retrodorsalis* having 3−14 in specimens from both the Nearctic and Palearctic possess other differences. These are the ratio of width to length of the pectoral fin, and diameter of the eye, and both are constant over its known range. According to Andriashev *G. retrodorsalis* tends to inhabit deeper waters, 100 − 300 m, than *G. viridis*, which is usually found in depths of less than 30 m. Our collections of *G. retrodorsalis* were from 8, 68, 68, and 250−300 m, the shallowest collection being the northernmost one from Axel Heiberg. Our collections of *G. viridis* are from 2 to 256 m, with 24 of 34 collections from 5 to 20 m in depth. Thus the morphological differences are accompanied by differences in depth preference and we are thus led to conclude that *G. retrodorsalis* is a valid species. Both species have been caught in the same trawl haul in Canada, Greenland, and USSR, although infrequently, so that they are sympatric and occasionally syntopic.

Our specimens agree reasonably well with the description of the holotype although it has fewer dorsal and anal rays, 85 and 75, as compared with 87 or more and 77 and more, respectively, in our specimens (including one-half the caudal rays in each).

Judging by the free dorsal fin ray pterygiophores we consider *G. retrodorsalis* to be the more derived of the two species.

A new vernacular is proposed, aurora pout, in allusion to its capacity to change color rapidly, and its northern distribution under the aurora borealis.

Distribution

The Axel Heiberg and Baffin Island records extend the known range over 2000 km W from eastern Greenland and provide the first known records for Canada. The species is also known from Jan Mayen, Spitsbergen, Novaya Zemlya, and localities throughout the Barents, Kara, and Laptev seas. Specimens are not yet known from the east Siberian, Chukchi, and Beaufort seas, so the species must be classified as discontinuous circumpolar. Its previous absence in Canadian collections was probably due to the lack of deep-water investigations in the Canadian Arctic. We have also identified numerous specimens from western Greenland.

Biology

Andriashev (1954) reports *G. retrodorsalis* from water (−1.8)−1.5 to 0.3°C and salinities of not less than 34.5‰. Our Axel Heiberg collection was from 0.1°C, one of the Frobisher collections from 0.32°C, and the Strathcona Sound one from 34.4‰. According to Andriashev it prefers muddy bottoms. Our Frobisher collections were from mud, clay, and gravel, the Strathcona Sound one from mud. Both species of *Gymnelus* were caught along with *Laminaria*, numerous ascidians and brittle stars at one station in Frobisher Bay. *Icelus bicornis* was caught with this species at Axel Heiberg, *Triglops nybelini*, *Leptagonus decagonus*, *Liparis fabricii*, *Leptoclinus maculatus*, and three species of *Lycodes* at Strathcona Sound.

Specimens collected from Frobisher Bay, Baffin Island, between 80 and 105 m on September 10 and 18 were composed of the following age groups and sizes:

Age	3	4	5
TL (mm)	93	107	122
Weight (g)	1.5	2.8	4.9

Andriashev (1954) reports females with 5−27 ripe eggs 4.0−4.5 mm in diameter in August to September, fewer eggs than *G. viridis* which usually has 10−15. The largest eggs in our collections were 4.0 mm in a 114-mm TL female taken August 11, 1970, in Frobisher Bay; six specimens caught September 10 and 18 had egg diameters between 0.6 to 1.1 mm. The available data suggest spawning about the end of August.

The specimen observed in an aquarium at Frobisher Bay swam in an undulating fashion. When resting it sat propped up on the anal and pectoral fins with the lower edges of the pectorals bent forward like feet.

Acknowledgments

We thank Jadwiga Aniskowicz for radiographing of numerous specimens for ray and vertebral counts. The staff of B.C. Research, Vancouver, generously donated specimens. The crews of M.V. *Calanus* and M.V. *Salvelinus* assisted in capturing specimens. Eugene Ruff collected the Alaskan specimens. Dr Jørgen Nielsen, University Zoological Museum, Copenhagen, kindly lent syntypes of *Lycodes pallidus squamiventer* and Dr A. P. Andriashev, Zoological Institute, Leningrad, a radiograph of the same species. We thank Referee No. 1 for pointing out that the original generic spelling *Gymnelus* is valid, whether or not it is grammatically correct, and that the subsequent emmendation, *Gymnelis*, is invalid. To all these persons we are grateful.

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Growth Patterns in Sand Lance, *Ammodytes dubius*, from the Grand Bank

G. H. WINTERS

Department of Fisheries and Oceans, Research and Resource Services, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Nfld. A1C 5X1

WINTERS, G. H. 1981. Growth patterns in sand lance, *Ammodytes dubius*, from the Grand Bank. Can. J. Fish. Aquat. Sci. 38: 841–846.

Growth patterns in sand lance (*Ammodytes dubius*) from the Grand Bank were examined using back-calculated lengths from otolith measurements. Lee's phenomenon of apparent change in growth rate with age is clearly demonstrated as is the phenomenon of growth compensation. Lee's phenomenon is explained by discriminatory mortality of the faster-growing sand lance whereas growth compensation is attributed to the differing ages of maturity of the initial faster-growing and slower-growing fish. The significant reduction in the standard deviation and the increasing degree of left skewness in length frequencies of older sand lance is explained by a combination of Lee's phenomenon and growth compensation acting together throughout the growth history of individual sand lance.

Key words: sand lance, growth, back-calculation, Grand Bank

WINTERS, G. H. 1981. Growth patterns in sand lance, *Ammodytes dubius*, from the Grand Bank. Can. J. Fish. Aquat. Sci. 38: 841–846.

La croissance du lançon (*Ammodytes dubius*) du Grand banc a été examinée à l'aide d'un calcul rétrospectif des longueurs à partir de mesures de l'otolithe. Le phénomène de Lee de modification apparente du taux de croissance avec l'âge est clairement démontré tout comme le phénomène de la compensation de croissance. Le phénomène de Lee s'explique par la mortalité sélective du lançon à croissance rapide alors qu'on attribue la compensation de croissance a l'âge de maturité variable du poisson selon qu'il a au départ une croissance plus rapide ou moins rapide. La réduction considérable de l'écart type et les fréquences croissantes de dissymétrie gauche au niveau de la longueur du lançon plus âgé s'expliquent par la conjugaison du phénomène de Lee et de la compensation de croissance pendant toute la croissance du lançon.

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SAND lance (*Ammodytes* spp.) are small semipelagic fish which are very common in coastal waters and offshore banks in the Northwest Atlantic area. Although commercially unimportant, they do occupy a strategic niche in the marine ecosystem as a major fodder species for such commercially important fish as haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*), silver hake (*Merluccius bilinearis*), yellowtail flounder (*Limanda ferruginea*), and American plaice (*Hippoglossoides platessoides*) (Scott 1968, 1973; Pitt 1973, 1976; Bowman and Langton 1978). In addition such large marine mammals as fin (*Balaenoptera physalus*) and humpback whales (*Megaptera novaengliae*) also feed heavily on sand lance (Overholtz and Nicholas 1979). In the Grand Bank area their role as an important prey species has probably been greatly enhanced in most recent years as a result of a substantial reduction in the abundance of capelin (ICNAF 1979) which has traditionally been the major prey species in that area. However, there is a paucity of published information on the biological and ecological charactersitics of sand lance populations on the Grand Bank. Meristic and morphometric studies have been conducted by Winters (1970) and Scott (1972), and larval and postlarval data have been reported by Dannevig (1918) and Frost (1938). Growth information for Grand Bank sand lance is lacking with the exception of mean population length data reported by Scott (1972). This paper presents new information on the growth patterns and intrinsic growth characteristics of sand lance in the Grand Bank area, based on analyses of back-calculated lengths from measurements of otolith radii.

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Methods and Materials

Random samples of sand lance from the southern and central areas of Grand Bank and mainly during April–June were obtained from incidental catches of that species by the research vessel *A. T. Cameron* during 1968–74. Standard trawling gear consisted of a No. 36 or No. 41-5 Yankee bottom trawl with a cod end liner of mesh size ranging from 15 to 30 mm. A total of 519 sand lance specimens were examined in detail after preservation in frozen brine. Lengths represent total lengths as measured from the tip of the man-

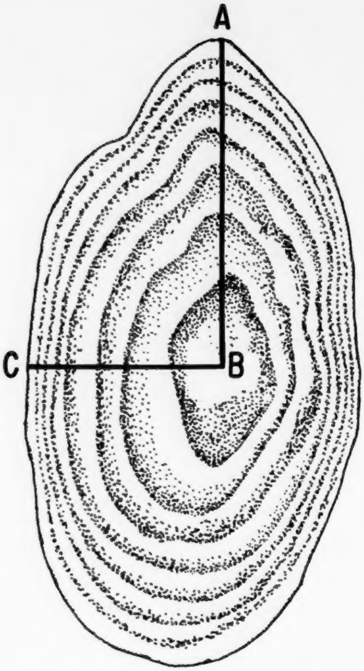

FIG. 1. Sketch of sand lance otolith indicating the manner in which otolith measurements were made. Line BC represents the measurement axis for annuli widths.

dible to the ventral lobe of the caudal fin, deflected backwards in a straight line with the body. Conversion of thawed lengths to fresh equivalents was based on regression analysis of several samples measured fresh and after thawing, indicating an adjustment factor of 1.03.

Age determinations were based on otolith interpretations according to the methodology of Macer (1966) and Scott (1968, 1973). All otoliths with poorly defined annuli (~6.5% of the total sample) were omitted in age determinations. Drawings of otoliths were made with a camera lucida attachment and with the anterior end (relative to the fish orientation) pointing to the top of the page and the proximal surface pointing upwards. A straight line, AB (Fig. 1), extending from the rostrum to the nucleus of the 1st year, was then drawn and the otolith radius was measured to the outside of each winter zone along a line BC at right angles to AB.

Results

OTOLITH RADIUS—FISH LENGTH RELATIONSHIP

The otolith radius measurements were grouped into class intervals of 5 AU (arbitrary units) and the average body length (mm) was plotted at the midpoint of each otolith radius interval. The results (Fig. 2) indicated that the relationship between fish length and otolith radius could be best fitted by two straight lines, and regression analyses indicated highly significant linear relationships that were then used as a basis for back-calculating lengths at annular radii. Scott (1973) also determined that a simple linear relationship could not adequately describe the otolith radius—fish length relationship of Scotian Shelf sand lance.

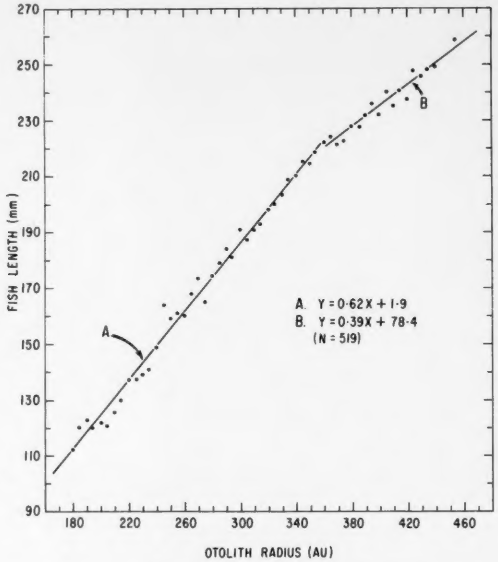

FIG. 2. Relationship between fish length and otolith radius for Grand Bank sand lance.

GROWTH PATTERNS OF SUCCESSIVE AGE-GROUPS

Age-specific back-calculated lengths at previous annuli for age-groups 1−8 are given in Table 1 and are illustrated graphically for age-groups 3−7 in Fig. 3. There is an initial divergence in the growth curves of successive age-groups followed by an apparent convergence after age-group 2. According to Zamakhaev (1965), this initial divergence and subsequent convergence of growth curves of successive age-groups is characteristic of Lee's phenomenon (Lee 1912) of apparent change in growth rate with age. A plot of back-calculated l_1 values against age (Fig. 4) clearly demonstrates this growth phenomenon in Grand Bank sand lance.

The subsequent convergence of initially diverging growth curves shown in Fig. 3 may also reflect the phenomenon of growth compensation between successive age-groups. To evaluate this growth pattern, back-calculated l_1 values of sand lance in age-group 5 were arranged in length groups of increasing magnitude in size, and their increments and subsequent growth patterns were compared. The results (Fig. 5) indicate a substantial influence of initial size on subsequent growth to the extent that large differences in size during the early growth period are substantially ameliorated by compen-

TABLE 1. Back-calculated lengths of sand lance as determined from the relationship given in Fig. 2.

Age at capture	Maturity	No. of specimen	Computed size at successive annuli (mm)							
			1	2	3	4	5	6	7	8
1	Imm.	4	103							
2	Imm.	99	100	133						
	Mat.	6	117	152						
3	Imm.	15	104	147	171					
	Mat.	92	113	161	189					
4	Imm.	12	100	145	172	191				
	Mat.	123	112	161	191	210				
5	Imm.	1	88	143	174	193	208			
	Mat.	102	106	153	187	211	221			
6	Mat.	45	96	147	180	206	221	230		
7	Mat.	17	85	132	172	203	222	233	239	
8	Mat.	3	86	117	156	188	209	230	244	252
	Mean		105	151	186	208	219	231	241	252

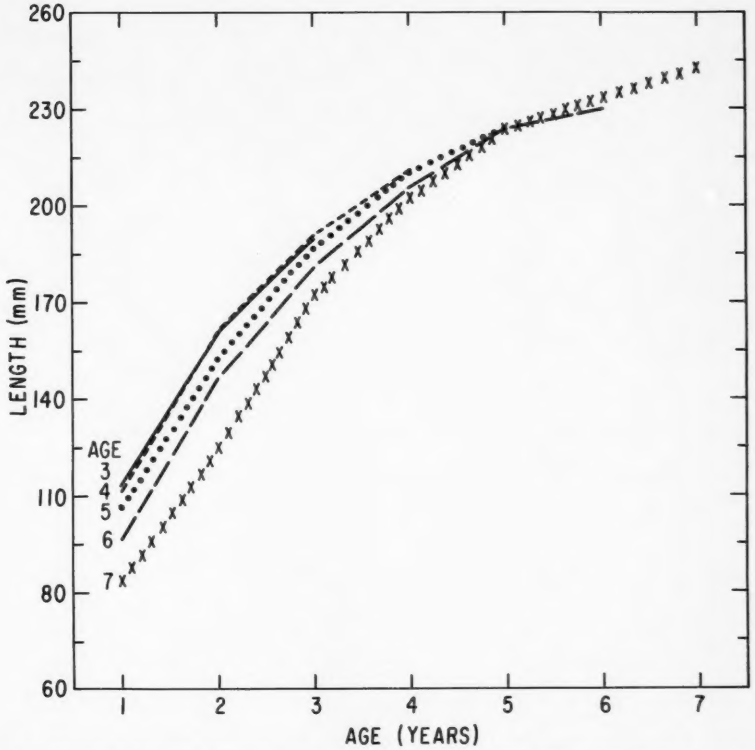

FIG. 3. Growth curves of successive age-groups of Grand Bank sand lance as determined from back-calculated lengths.

satory growth throughout the subsequent growth history of the individual fish. Such compensatory growth mechanisms will of course have confounding effects on density-dependent growth processes.

DESCRIPTION OF GROWTH CURVE

The age-length relationship of Grand Bank sand lance was derived by averaging the back-calculated lengths of all fish at

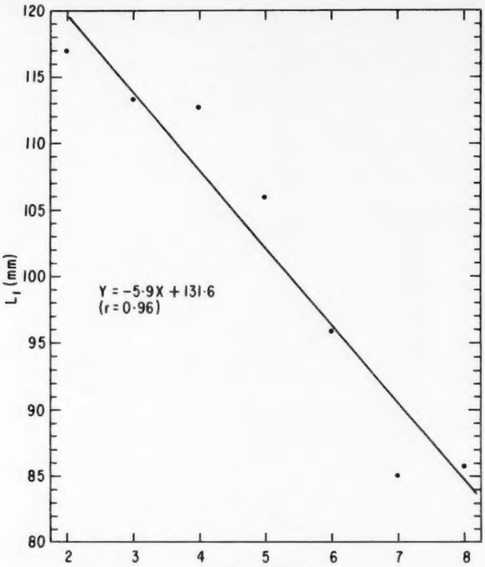

FIG. 4. The relationship between back-calculated length at the end of the first year (l_1) and age.

each age. These mean back-calculated lengths thus represent the average of the former sizes of those fish that have survived to the sampling age and according to Ricker (1969) are more representative of the true population growth rate than the empirical length-at-capture relationship when sampling is biased. The presence of "Lee's" phenomenon (Fig. 3 and 4) may be attributed to either biased sampling or selective mortality but, given the small mesh size of the cod end liner (15−30 mm) and a natural mortality rate increasing with age (Winters unpublished data), it is concluded that selective mortality by size is the major component of Lee's phenomenon for Grand Banks sand lance. Therefore I have used empirical age-specific lengths to describe the growth of Grand Bank sand lance.

The growth rate data derived in the above manner were analyzed according to the von Bertalanffy (1938) growth model:

$$l_t = l_\infty\ (1 - e^{-k(t-t_0)})$$

where l_t is the mean length at age t years, l_∞ is the asymptotic length, k is a constant determining the rate of change in length increments, and t_0 is the theoretical age at zero length. The growth curve was fitted using the method of Allen (1966).

The von Bertalanffy growth model provides an adequate description of the growth of Grand Bank sand lance (Fig. 6). The l_∞ estimate is within the upper extreme of empirical lengths of Grand Bank sand lance and may therefore be considered a realistic estimate of maximum length from that area. For comparative purposes growth curves of *Ammodytes dubius* populations from Emerald Bank and Banquereau Bank (Scott 1973) are also shown in Fig. 6. The Grand Bank population has a growth rate which is intermediate between the two Scotian Shelf populations of sand lance.

TEMPERATURE AND GROWTH

In addition to the intrinsic growth factors which have been shown above to have a significant and predictable influence on life history growth patterns, environmental factors may also exert considerable influence on fish growth.To evaluate this environmental influence on growth, I selected surface temperature anomalies in the Grand Bank area as the environmental signal and these have been plotted against l_1 values (back-calculated) for the 1963−69 year-classes appropriately adjusted for Lee's phenomenon (to age-groups 3) from the relationship shown in Fig. 6. The results (Fig. 7) indicate a highly significant positive relationship between temperature and l_1 growth of Grand Bank sand lance.

Discussion and Conclusions

The occurrence of Lee's phenomenon in sand lance is probably related to the higher maturation rate of faster-growing fish that subjects these individuals earlier and in greater frequency to the stresses associated with reproduction. This discriminatory mortality rate of the faster-growing individuals probably also accounts for the convex nature of the catch curve of sand lance (Winters unpublished data), implying increasing mortality with age. Jones (1958) has shown that when mortality increases linearly with size (age), the frequency distribution of the lengths of the survivors in the year-class remains normal, and the standard deviation does not change. However, as noted by Ricker (1969), growth compensation may produce a significant reduction in the variance in length with age and also a tendency for the age-specific length frequencies to be more and more skewed to the left in the older age-groups. Figure 8 depicts the length frequencies for *A. dubius* for the period 1968−74 (immatures and matures combined). The bimodality of lengths in age-group 2 reflects the substantial intermixture of mature and immature lance which have different growth rates. The substantial reduction in the standard deviations and the increasing degree of left skewness with increasing age supports the explanation of higher mortalities associated with faster-growing individuals as a basis for explaining Lee's phenomenon in sand lance. A similar occurrence of Lee's phenomenon in capelin (*Mallotus villosus*) was explained by Winters (1974) on the basis of increased spawning mortality of the larger, faster-growing individuals as indicated by a comparison of age-specific lengths of pre- and post-spawning capeliin from the Grand Banks area.

The growth compensation that is evident in the subsequent growth patterns of a cohort of sand lance (Fig. 6) also results from the differing ages of maturity of the initial faster-growing and slower-growing fish. This is reflected in the larger l_1 values associated with mature fish than with immature fish of the same age-group (Table 1). Thus the retardation in growth rate normally associated with the onset of first maturation occurs earlier in the initially faster-growing indi-

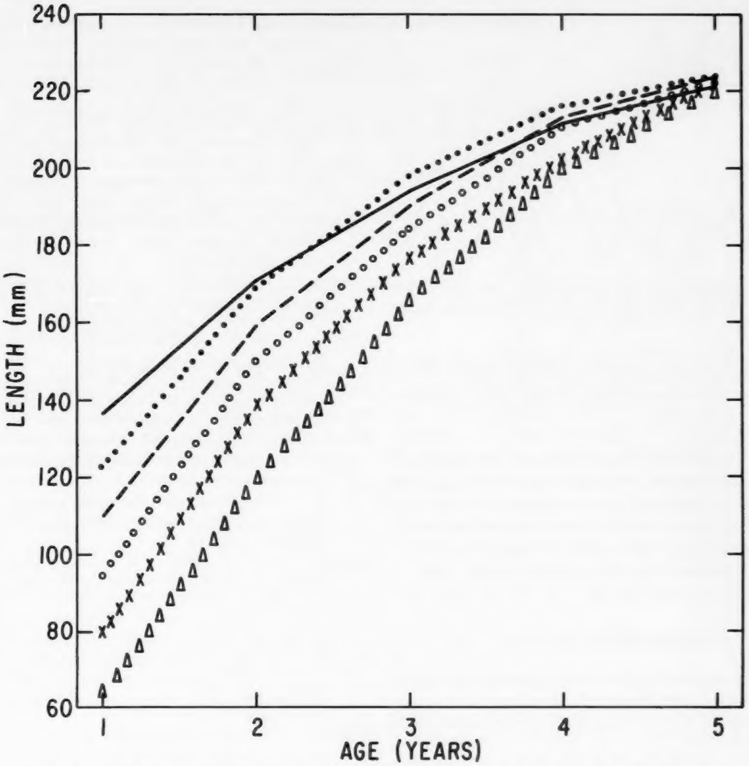

FIG. 5. Life history growth patterns of 5-yr-old sand lance in relation to size in the 1st year.

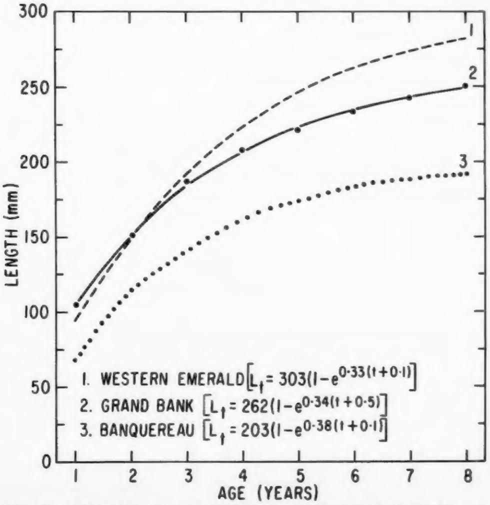

FIG. 6. Growth curves of sand lance as determined from the von Bertalanffy growth model.

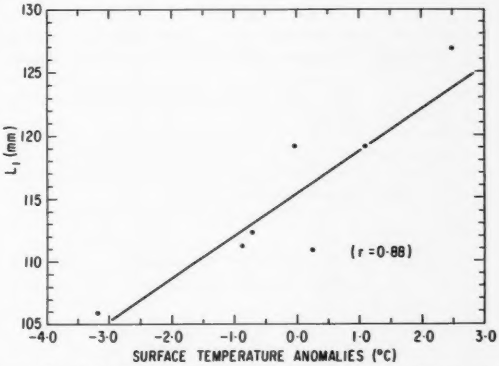

FIG. 7. Relationship between 1st-year growth (l_1) of sand lance and surface temperature anomalies on the Grand Bank.

viduals, resulting in a convergence of their growth curve with the slow-growing, later-maturing individuals of a cohort.

Scott (1973) attributed the lower growth rate of sand lance on Banqeureau than on Emerald Bank to the lower bottom temperatures characteristic of the Banquereau area. Compara-

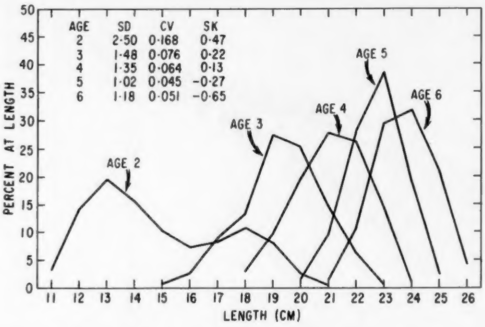

FIG. 8. Age-specific length frequency of Grand Bank sand lance as obtained from research vessel sample collections 1968−74. SD = standard deviation, CV = coefficient of variation, SK = degree of skewness).

ble data are lacking for the Grand Bank area, although both surface and bottom temperatures are generally lower in that area than on the Scotian Shelf. Nevertheless in certain areas such as the southern slope, water temperatures are fairly warm in relation to Banquereau Bank which is subject to the influence of the cold Labrador Current, and this may account for the intermediate growth rate of the Grand Bank sand lance.

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A Probability Transition Matrix Model for Yield Estimation in Fisheries with Highly Variable Recruitment

WAYNE M. GETZ

Departments of Entomological Sciences and Plant Pathology, University of California, Berkeley, CA 94720, USA

AND GORDON L. SWARTZMAN

Center for Quantitative Science in Forestry, Fisheries and Wildlife, University of Washington, Seattle, WA 98195, USA

GETZ, W. M., AND G. L. SWARTZMAN. 1981. A probability transition matrix model for yield estimation in fisheries with highly variable recruitment. Can. J. Fish. Aquat. Sci. 38: 847–855.

This paper addresses the problem of estimating the yield variability that is encountered when managing a fishery that exhibits high recruitment variability. A model is developed that combines elements of a discrete time, age-structured matrix model, a Beverton–Holt continuous time harvest yield model, and a stock–recruitment Markov probability transition matrix model. The model calculates the mean and variance of the stock levels and fishery yields associated with given fishing effort levels, and this type of information can be used to examine the response of the fishery to the implementation of various management strategies. The model is used to contrast the effects of recruitment variability in three fisheries, viz. the South African anchovy (*Engraulis capensis*) purse-seine fishery, the southern New England yellowtail flounder (*Limanda ferruginea*) trawl fishery, and the Georges Bank haddock (*Melanogrammus aeglefinus*) fishery.

Key words: stochastic population dynamics, fisheries yield estimation model, variable recruitment, anchovy, yellowtail flounder, haddock

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Les auteurs s'attaquent à la difficulté d'estimer la variabilité du rendement d'une espèce pêchée dont le recrutement est très variable. Ils mettent au point un modèle qui réunit les éléments d'une matrice des âges à des temps discrets, un modèle du rendement de récolte continue à la Beverton et Holt et une matrice de transition markovienne du recrutement des stocks. Le modèle permet de calculer la moyenne et la variance des stocks et des rendements de la pêche pour un effort donné de pêche, et ce type d'information peut servir à examiner la réaction de l'espèce à la mise en place de diverses formes de gestion. Le modèle sert à mettre en valeur les effets de la variabilité du recrutement dans trois pêcheries, c'est-à-dire, celle de l'anchois du Cap (*Engraulis capensis*), en Afrique du Sud, à la senne coulissante; celle de la limande à queue jaune (*Limanda ferruginea*), dans le sud de la Nouvelle-Angleterre, au chalut; et celle de l'églefin (*Melanogrammus aeglefinus*), sur le banc George.

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ALMOST all marine fish stocks experience a wide range of variability in the strength of consecutive year-classes (Cushing 1973), yet deterministic stock–recruitment relationships predominate in the application of mathematical models to analyzing fisheries management problems and computing maximum sustainable yields (e.g. Beverton and Holt 1957; Clark 1976; Getz 1980a, 1980b; Ricker 1975). This problem of variability needs to be squarely faced if fisheries management models are going to play a role in rehabilitating the many economically important fisheries that have collapsed or are presently in the process of collapsing. Variability in recruitment is the primary source of uncertainty in most fisheries and is the area that we will focus on in this paper. The

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general question of exploiting a fishery in an uncertain environment using a one-dimensional stochastic differential equation model has been addressed by May et al. (1978).

There have been several recent studies that have explored the problem of variability in recruitment and attempted to uncover the source of this variability. These include inter alia single-species, age-structured models where recruitment has been correlated with such environmental variables as temperature, Eckmann transport, or river flow (Nelson et al. 1976; Leggett 1977; Sissenwine 1977; Stevens 1977), a multispecies simulation model which links year-class strength to interspecific competition via predation (Andersen and Ursin 1977), a Ricker stock–recruitment model in which one of the parameters of the stock–recruitment relationships is assumed to be drawn from an a priori determined probability distribution (Walters 1975), larval transport models that assume density-dependent survival (Eraslan et al. 1976), and larval feeding models based on prey patchiness (Vlymen 1977).

In addition to recruitment variability, a management model of general applicability needs to account for the fact that the stocks of most commercial fisheries are relatively long-lived and are often fecund and fished over a number of years of their life cycle. Furthermore, the age structure of a fish stock may frequently change with the development of a fishery as happens in the often observed juvenation process (Bakkala et al. 1979). Finally, age (size) structure is important as fishery gear is most often size selective and fecundity is age (size) specific.

The model presented in this paper combines elements of a discrete time, age-structured Leslie matrix population model (Leslie 1945) with a Beverton–Holt continuous time harvest yield model (see Getz 1980a, b) and a stock–recruitment Markov probability transition matrix model. The model separates the parent stock and each age-class into an appropriate choice of population levels and produces as output a vector that assigns a probability of finding the parent stock and the number of individuals in each age-class at each of the given levels. The model also calculates the mean and variance associated with the stock levels and the fisheries yield at a given (fishing) effort level.

Walters (1975) has previously suggested the use of a Markov transition matrix approach in dealing with recruitment variability but rejected it for his problem (Skeena River sockeye salmon (*Oncorhynchus nerka*) fishery) on the grounds that insufficient data were available to estimate the stock–recruitment transition probabilities. This is a problem that we have had to face in all the fisheries we have examined in detail. We believe, however, that the transition matrix approach provides a more natural, less biased, utilization of the raw stock–recruitment data than the imposition of a preconceived stock–recruitment relationship on a particular fishery. Furthermore, a sensitivity analysis on the elements of the transition matrix is more likely to reveal the consequences of high variability on managed and trended fisheries than a sensitivity analysis on a stock–recruitment relationship with a predetermined form as the former contains many more parameters than the latter.

Besides presenting a general description of our model, we present results that we have obtained for three major and quite different fish stocks, viz. an anchovy (*Engraulis capensis*) purse-seine fishery, a yellowtail flounder (*Limanda ferruginea*) trawl fishery, and a haddock (*Melanogrammus aeglefinus*) trawl fishery, and we discuss these results in some detail.

Mathematical Model

DEFINITION OF SYMBOLS

c_i	Fraction of sexually mature fish in age-class i
$E(\cdot)$	Expected value (mean) of argument
i	Age-class index ($i = 1, \ldots, n$)
j	Index of subdivision of population numbers in each age-class for the discrete probability distributions ($j = 1, \ldots, n$)
j_i	The index j above as it pertains to the ith age-class (as in j_1, j_{n-1}, etc.)
j_s	Index of subdivision of stock biomass levels for the discrete probability distributions ($j_s = 1, \ldots, m_s$)
k	Index for time in integer years
m	Number of subdivisions for age-class distributions
m_s	Number of subdivisions for adult stock biomass discrete distribution
n	Number of age-classes in the model
$\mathbf{P}_i(\cdot)$	Vector of probabilities (discrete probability distribution) for population levels in age-class i
$p_{ij}(\cdot)$	The jth element of $\mathbf{P}_i(\cdot)$ above
$\mathbf{P}_s(\cdot)$	Vector of probabilities (discrete probability distribution) for adult stock biomass levels
$p_{sj}(\cdot)$	The jth element of $\mathbf{P}_s(\cdot)$
q_i	Catchability coefficient for age-class i
r	The age at which a fish stock is recruited to the fishery
$s(\cdot)$	Stock biomass level
$\hat{s}$	Maximum stock biomass level
t	Time (years)
t_s	Time spawning season begins as a proportion of the year that has passed, i.e. $0 \leq t_s < 1$
f	$[0, f]$ is the portion of each year that is open to fishing
$\mathbf{T}$	Stock–recruitment transition matrix of elements t_{ij} ($i = 1, \ldots, n$ and $j = 1, \ldots, m_s$) (see below)
t_{ij}	Denotes the probability that stock biomass level j will give rise to recruits r years later at recruit level i (or using indices defined above $t_{ij} = t_{i_i j_s}$)
$V(\cdot)$	Variance of arguments
$v(t)$	Fishing intensity over time
v	Constant fishing intensity
v_k	Constant fishing intensity during year k
$v_{\max}$	Fishing intensity at which yield is maximal
w_i	Average biomass of an individual fish in age-class i
x_i	Number of fish in age-class i (a random variable)
$\hat{x}_i$	Maximum number of fish in age-class i
Y_k	Yield in year k (a random variable)
α_i	Natural mortality rate for fish in age-class i
σ	Standard deviation of yield

MODEL

Assume that the stock of a fishery can be divided into n year-classes (as in fisheries with recognizable cohorts although other age-class divisions can be considered), where x_i,

$i = 1, \ldots, n$, is used to represent the numbers of individuals in the ith age-class. Because accurate estimates of mortality rates and catchability parameters are usually difficult to obtain, it will be assumed that these rates and parameters are constant over the year, although they can be made time-dependent if required. Assume than that x_i is reduced throughout the year by a natural mortality rate α_i and during the fishing season by an additional fishing mortality rate $q_i v(t)$, where $v(t)$ is the fishing intensity as a function of time and q_i is the catchability coefficient for age-class i.

If we choose to begin monitoring the population at the beginning of the harvesting (fishing or open) season, which runs for a portion $\hat{t}$ say, of the year, i.e. $0 < \hat{t} < 1$, and we assume that the fishery is closed for the balance of the year (closed season, possibly during spawning) then, using Beverton and Holt's dynamic model, we have during the $k + 1$th year that

$$(1) \quad \frac{dx_i}{dt} = -(\alpha_i + q_i v_k)x_i \qquad \text{for } i = 1, \ldots, n \text{ and } t \epsilon [k, k + \hat{t}]$$

$$\frac{dx_i}{dt} = -\alpha_i x_i \qquad \text{for } i = 1, \ldots, n \text{ and } t \epsilon [k + \hat{t}, k + 1]$$

where we have assumed that the fishing effort $v(t)$ has the constant value v_k over the $k + 1$th fishing season (again this assumption of constancy can be weakened if necessary).

Consider the new recruits to the fishery at the beginning of the $k + 1$th year, i.e. the year-class $x_1(k)$. Also consider the spawning stock that spawned these recruits during the $(k - r)$th year, where r is the number of years that recruitment of new stock to the fishery is delayed after spawning, and let $s(k - r - 1 + t_s)$ denote the stock's biomass level at the beginning of the kth spawning season, where $t_s \epsilon [0, 1]$ denotes the time that the spawning season begins each year. Depending on the nature of the available data, the biomass of the spawning stock may have been identified with the total biomass of the fishery or more realistically with the sum of the biomass of all fecund year-classes and an appropriate proportion of the biomass of all partially fecund year-classes, i.e.

$$(2) \quad s(k-r-1+t_s) = \sum_{i=1}^{n} c_i w_i(k-r-1+t_s) \, x_i(k-r-1+t_s)$$

where $w_i(t) > 0$ is the average biomass of an individual in age-class i at time t and $c_i \epsilon [0, 1]$ is the proportion of fecund individuals in the ith age-class.

Stock–recruitment data pertaining to a particular fishery typically consist of points in the $(s(k - r - 1 + t_s), x_1(k))$ plane for various years (k). This plane can be partitioned by choosing upper bounds $\hat{s}$ and $\hat{x}_1$ say, based on the data for the stock and recruit levels, respectively, and dividing the intervals $[0, \hat{s}]$ and $[0, \hat{x}_1]$ into m_s and m equal subdivisions, respectively. The assumption of equal subintervals can be omitted if necessary and the most appropriate values for m_s and m will depend on the amount of data available and their grouping in the stock–recruit plane. Too few subdivisions will result in excessively coarse estimates of the mean and variance associated with the stock and yield distributions while many subdivisions are only justified when large stock–recruitment data bases are available and will have the effect of increasing the time of the simulation run (although the number of year-classes n is more critical in this respect). Estimates of the probability that the recruits $x_1(k)$ will be in the j_1th subdivision (of m possible subdivisions), given that the parent stock level $s(k - r - 1 + t_s)$ is in its j_sth subdivision (of m_s possible subdivisions), can be made on the basis of the actual number of stock–recruit data points that fall into the rectangle

$$\left[\left(\frac{j_s - 1}{m_s}\right)\hat{s}, \left(\frac{j_s}{m_s}\right)\hat{s}\right] \times \left[\left(\frac{j_1 - 1}{m}\right)\hat{x}_1, \left(\frac{j_1}{m}\right)\hat{x}_1\right]$$

in the $(s(k - r - 1 + t_s), x_1(k))$ plane. These probabilities, $t_{j_1 j_s}$, $j_1 = 1, \ldots, m$ and $j_s = 1, \ldots, m_s$, can be regarded as elements of a matrix $\mathbf{T}$ which then represents a Markov-type stock–recruitment transition matrix.

Because we have assumed that the new recruits at time k, i.e. $x_1(k)$, will fall into one of m subdivisions of $[0, \hat{x}_1]$, it follows from the Beverton–Holt equations (Equation (1)) that the second age-class will fall into one of m subdivision of $[0, \hat{x}_2(k + 1)]$ where

$$\hat{x}_2(k + 1) = e^{-(\alpha_1 + q_1 v_k \hat{t})}\hat{x}_1(k).$$

Similarly it follows that

$$(3) \quad \hat{x}_{i+1}(k + 1) = e^{-(\alpha_i + q_i v_k \hat{t})}\hat{x}_i(k) \qquad i = 1, \ldots, n - 2$$

whenever $\hat{x}_i(k)$ is known and that

$$(4) \quad \hat{x}_n(k + 1) = e^{-(\alpha_{n-1} + q_{n-1} v_k \hat{t})}\hat{x}_{n-1}(k) + e^{-(\alpha_n + q_n v_k \hat{t})}\hat{x}_n(k)$$

where the last term is a consequence of the fact that all individuals greater than $n = 1$ yr of age are also regarded as belonging to the nth year-class. Note that only $\hat{x}_1(k)$ is constant for all k (its value is set by the initial stock–recruitment data)

$$(5) \quad \hat{x}_1(k) = \hat{x}_1 \qquad \text{for all } k = 0, 1, 2, \ldots$$

while initial values $\hat{x}_i(0)$ $i = 2, \ldots, n$ must be specified before Equations (3)–(5) can be used to generate $x_i(k)$, $i = 2, \ldots, n$ for all $k = 1, 2, 3, \ldots$.

Finally to complete the model we need to associate a probability vector $\mathbf{P}_i(k)$, representing the probability distribution of $x_i(k)$ over the m subdivisions of $[0, \hat{x}_i(k)]$ for $i = 1, \ldots, n$ and $k = 1, 2, 3, \ldots$, with the ith age-class of the population at time k. Thus the jth element of $\mathbf{P}_i(k)$, denoted $p_{ij}(k)$, $j = 1, \ldots, n$, is the probability that the ith age-class is in the subdivision $\left[\left(\frac{j-1}{m}\right)\hat{x}_i(k), \left(\frac{j}{m}\right)\hat{x}_1(k)\right]$ in year k. This must also be done for the stock size s using a probability vector $\mathbf{P}_s$ with elements p_{sj}, $j = 1, \ldots, m_s$ that denote the probability that the stock level is in the interval $\left[\left(\frac{j-1}{m_s}\right)\hat{s}, \left(\frac{j}{m_s}\right)\hat{s}\right]$. The vectors $\mathbf{P}_i(k)$ and $\mathbf{P}_s(k - r - 1 + t_s)$ are calculated using an algorithm described in the Appendix and require the specification of initial distributions

$\mathbf{P}_i(0)$, $i = 1,\ldots, n$ and $\mathbf{P}_s(t_s - j)$, $j = 1,\ldots, r$, where the latter does not need to be specified if $r = 0$. This algorithm is based on the assumption that the recruit probability distributions $\mathbf{P}_1(k)$, $k = 0, 1, 2,\ldots$ are independently generated. The validity of this assumption depends on the amount of variability in the stock–recruitment data and is reasonable only when variability is high. This assumption, however, is not too restrictive as, in the case of low variability, deterministic models provide the most suitable approach.

If we take the midpoint of a subdivision as the nominal value of the variables $x_i(k)$ or $s(k + t_s)$ whenever they are considered as belonging with a certain probability to that subdivision, then the mean and variance associated with each of the separate year-classes or the stock level as easily evaluated. For example, the mean stock level $E(s)$ at time $k + t_s$ is

$$(6)\quad E(s(k + t_s)) = \sum_{j=1}^{m_s} \left(\frac{j - 0.5}{m_s}\right)\hat{s}p_{sj}(k + t_s)$$

while the variance $V(s)$ at time $k + t_s$ is given by

$$(7)\quad V(s(k + t_s)) = \sum_{j=1}^{m_s} \left(\frac{j - 0.5}{m_s}\right)^2 \hat{s}^2 p_{sj}(k + t_s) - E(s(k + t_s))^2.$$

Associated with each harvesting season is a biomass yield whose mean and variance can be estimated from the output of the model. From Equation (1), it follows that if $x_i(k)$ is given a value at time k then the yield Y_{k+1} during the k + 1th year will be

$$(8)\quad Y_{k+1} = v_k \sum_{i=1}^{n} q_i x_i(k) \int_k^{k+f} w_i(t) e^{-(\alpha_i + q_i v_k)t} dt$$

where $w_i(t)$ is the biomass function introduced in Equation (2) above. Assuming $w_i(t)$ is a constant it follows that the mean yield $E(Y_{k+1})$ will be

$$(9)\quad E(Y_{k+1}) = v_k \sum_{i=1}^{n} w_i q_i (1 - e^{-(\alpha_i + q_i v_k)f}) x_i(k) \sum_{j=1}^{n} \left(\frac{j - 0.5}{n}\right) p_{ij}(k)/(\alpha_i + q_i v_k).$$

If $w_i(t)$ is not constant, as will be the case if $w_i(t)$ is derived from a von Bertalanffy growth function, then Equation (8) will have a more complex form that involves four separate terms (see, for example, Getz 1980b). As in Equation (7) the variance of Y_{k+1} can also be calculated and its positive square root (i.e. the standard deviation) used to obtain some insight into actual yield variability.

The model can also be used to generate mean sustainable yield and stock levels and associated variance levels for a preselected fishing effort by allowing the simulation to run for several years until an equilibrium solution is obtained. This will not always necessarily happen and even if an equilibrium solution is obtained it may not necessarily be unique. This type of analysis, if carried out for a range of fishing efforts, should be useful in revealing the long-term potential of the fishery to provide sustained yields and the variability involved.

EXAMPLES

Description of data sets — In this section we will discuss the application of our probability transition matrix model to three fisheries. The results obtained represent only a preliminary analysis of the fisheries involved and should be regarded more as a demonstration of how the model can be directly utilized to examine the yield potential and variability in a fishery than as providing a substantive analysis of the fisheries themselves.

The three fisheries are (i) the purse-seine anchovy fishery situated off the western Cape Coast of South Africa; (ii) the southern New England yellowtail flounder trawl fishery; (iii) the Georges Bank haddock trawl fishery. For each of these fisheries, data from available publications were used to estimate the parameters needed for the model (anchovy, Getz 1980a, b; flounder, Sissenwine 1974, 1977; haddock, Grosslein and Hennemuth 1973). This was deemed to be adequate for our purposes, but if a more comprehensive analysis of any of the fisheries was to be undertaken it would be preferable to reexamine the raw and updated data directly, especially to ensure that the stock–recruitment data are in a form that is compatible with the requirements of the model. The data we used are listed in Tables 1 and 2.

Although five year-classes were used to analyze all three fisheries, recruitment in the flounder and haddock fisheries is delayed by 1 yr after spawning (Table 1) so that in these two fisheries the first year-classes contain individuals in their 2nd yr of existence while the fifth year-classes contain individuals in their 6th yr of existence and beyond. We also note that although the number of recruits to the anchovy fishery is approximately three orders of magnitude greater than the number of recruits to the flounder and haddock fisheries, the actual biomass of the spawning stocks are not dissimilar because the anchovy are subject to a significantly higher mortality rate and are ~25 times less massive than the flounder and 50 times less massive than the haddock. The anchovy fishery possesses a relatively more vigorous and robust stock–recruitment relationship (see transition matrices in Table 2) and is consequently almost 8 times more productive on average than the flounder and haddock fisheries. This value of 8 is obtained from our analysis as portrayed below.

All three fisheries have markedly different stock–recruitment behavior. The anchovy exhibit poor to moderate recruitment at low stock levels, moderate recruitment at intermediate stock levels, and strong recruitment at high stock levels (Table 2). In contrast, the flounder exhibit poor recruitment at both low and high stock levels and variable (poor to strong) recruitment at intermediate stock levels while the haddock exhibit variable recruitment at low and intermediate stock levels and poor recruitment at high stock levels.

The data sets considered here are among the best organized of available fisheries data that have reasonably extensive stock records. The North Pacific Ocean halibut (*Hippoglossus stenolepsis*) fishery (Southward 1968) has comparable data sets although the large number of age-classes used by Southward to describe the fishery (about n = 15) will need to be reduced (e.g. by combining pairs of consecutive age-classes)

TABLE. 1. Parameter values used in the simulation runs.

	Parameters	Anchovy	Flounder	Haddock
n	No. of age-classes	5 (age-classes 1–5)	5 (age-classes 2–6)	5 (age-classes 2–6)
r	No. of years that recruitment is delayed after spawning	1	2	2
m	No. of age-class subdivisions	7	6	7
m_s	No. of parent stock subdivisions	8	8	6
$\hat{x}_1$	Maximum no. of recruits	105×10^9	100×10^6	120×10^6
$\hat{s}$	Maximum parent stock biomass (kg)	200×10^6	384×10^6	120×10^6

		Age-classes					
Parameters	Stock	1	2	3	4	5	6
Mortality rates α_i (yr^{-1})	Anchovy	0.80	0.80	0.80	0.80	0.80	[b]
	Flounder	[a]	0.20	0.10	0.10	0.10	0.10
	Haddock	[a]	0.50	0.22	0.22	0.22	0.22
Catchability coefficient q_i (fraction of year-class recruited to the fishery)	Anchovy	0.24	0.36	0.42	1.00	1.00	[b]
	Flounder	[a]	0.00	0.45	0.80	0.95	1.00
	Haddock	[a]	0.37	1.00	1.00	1.00	1.00
Fecundity coefficient c_i (fraction of sexually mature individuals in each age-class)	Anchovy	0.50	1.00	1.00	1.00	1.00	[b]
	Founder	[a]	0.06	0.53	0.87	1.00	1.00
	Haddock	[a]	0.00	0.40	0.78	1.00	1.00
Weight coefficients w_i (g)	Anchovy	2.40	8.30	14.8	20.6	25.1	[b]
	Flounder	[a]	160	330	510	630	810
	Haddock	[a]	320	500	910	1300	1800

[a] These entries were not required as the classes involved were not yet recruited to the fishery.

[b] These entries were not required as only five age-classes were considered in the anchovy fishery model.

to apply our model as the computation time required when $n > 7$ can run into hours on a CDC 6400. The model also could be usefully applied to the analysis of marine mammal stocks such as the Pribilof Island fur seal stock (Chapman 1964).

For each fishery the model was run from a nominal set of initial conditions until an equilibrium point (anywhere from 5 to 25 yr) was reached. This was done over a range of fishing effort values based on the actual efforts observed for that fishery. For each value of the effort the corresponding mean stock and yield levels and their associated standard deviations were calculated (c.f. Equations (6), (7), and (9) above). These results enabled us to plot yield–effort curves for all three fisheries and these curves are presented in Fig. 1–3 below. In all three figures the curves displaying the mean yield as a function of effort are bracketed by curves representing one and two standard deviations on either side of the mean. These latter curves provide us with a useful measure of the variance associated with corresponding yields even though the actual distributions and hence exact probabilities of falling within one or two standard deviations of the mean are not known. We do at least know from Chebyshev's inequality (see e.g. Meyer 1966) that under sustainable yield conditions at least 75% of all observed yields will fall within two standard deviations of the mean and that this estimate is extremely conservative if the yield distribution is unimodal (e.g. ~95% of observed yields would be within two standard deviations of the mean yield if the sustainable yield level was normally distributed). It should also be noted that because yields can only be positive, it is likely that a greater proportion of events will be bracketed between the mean and the two standard deviation lower bound than the mean and the two standard deviation upper bound.

Before proceeding to discuss our analysis we would like to point out that the estimates of the elements of the stock recruitment probability transition matrices listed in Table 2 are crude and should not be regarded as highly reliable. Despite this weakness in the data, however, it will be seen below that analysis of the model provides some interesting insights into the role of stock–recruitment variability in the fish stocks considered, especially when comparing the response of the three fisheries considered. A more detailed analysis should be based on a thorough examination of the raw data (not usually available in the literature) to ensure its reliability and compatibility with our modeling approach. Sensitivity analysis can also be carried out to evaluate the robustness of the results obtained.

Results

From Fig. 1–3 we see that all three fisheries rise to a maximum with the anchovy and haddock fisheries experiencing a gradual decline in yields beyond their maxima while the flounder fishery exhibits a rapid and dramatic drop beyond its maximum. The mean and standard deviations associated with the maximum sustainable yields and the corresponding coefficient of variation and fishing effort are given in Table 3. The coefficient of variation of yield provides a useful comparative measure of variability among the three fisheries and is plotted in Fig. 4 as a function of normalized effort (i.e. effort as a fraction of the effort corresponding to the maximum mean sustainable yield).

Note that the interpretation of v differs from fishery to fishery and that an effort level of $v = 1$ in one fishery does not directly translate into $v = 1$ in another fishery. In fact,

TABLE. 2. Stock–recruitment probability transition matrices. The transition matrix elements t_{ij} represent the probability that a stock biomass in subdivision j ($j \leq m_s$) will result in the number of recruits being in subdivision i ($i < m$). Note that each column of elements represents a probability distribution of recruitment frequencies corresponding to the indicated stock level (j).

Anchovy

High	7(=m)	0.00	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	6	0.00	0.05	0.10	0.10	0.15	0.20	0.25	0.25
	5	0.05	0.10	0.15	0.25	0.30	0.35	0.35	0.35
Recruitment levels (i)	4	0.15	0.20	0.35	0.30	0.25	0.20	0.20	0.20
	3	0.15	0.40	0.25	0.25	0.20	0.15	0.10	0.10
	2	0.45	0.15	0.10	0.05	0.05	0.05	0.05	0.05
Low	1	0.20	0.05	0.00	0.00	0.00	0.00	0.00	0.00
	i / j	1	2	3	4	5	6	7	8 (=m_s)

Low ← Stock levels (=j) → High

Flounder

High	6(=m)	0.00	0.00	0.10	0.05	0.20	0.00	0.00	0.00
	5	0.00	0.00	0.05	0.26	0.20	0.10	0.05	0.00
	4	0.00	0.00	0.05	0.25	0.20	0.10	0.15	0.00
Recruitment levels (i)	3	0.00	0.15	0.15	0.15	0.20	0.15	0.40	0.20
	2	0.50	0.40	0.30	0.15	0.20	0.40	0.20	0.40
Low	1	0.50	0.45	0.35	0.15	0.00	0.25	0.20	0.40
	i / j	1	2	3	4	5	6	7	8 (=m_s)

Low ← Stock levels (j) → High

Haddock

High	7(=m)	0.10	0.10	0.05	0.12	0.00	0.00
	6	0.10	0.10	0.05	0.12	0.00	0.00
	5	0.10	0.05	0.25	0.12	0.10	0.00
Recruitment levels (i)	4	0.10	0.10	0.05	0.12	0.20	0.00
	3	0.10	0.25	0.50	0.12	0.30	0.30
	2	0.20	0.25	0.05	0.40	0.30	0.30
Low	1	0.30	0.15	0.05	0.12	0.10	0.40
	i / j	1	2	3	4	5	6 (=m_s)

Low ← Stock levels (j) → High

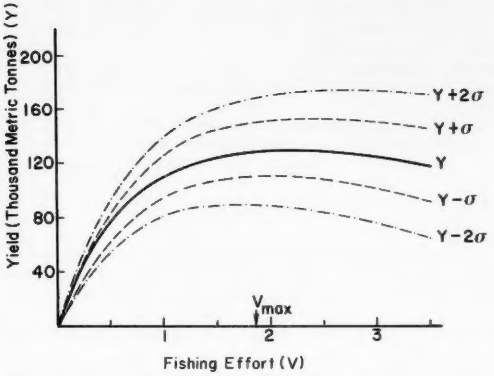

FIG. 1. Sustainable yield levels for the South African west coast anchovy fishery.

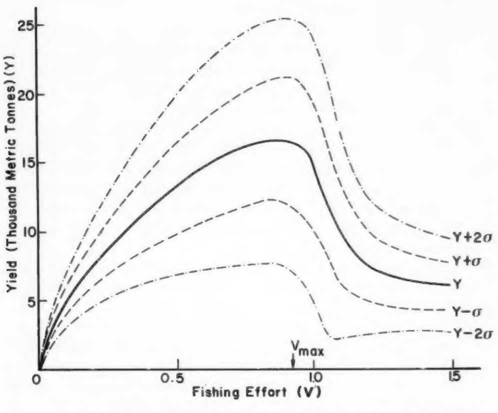

FIG. 2. Sustainable yield levels for the Southern New England yellowtail flounder fishery.

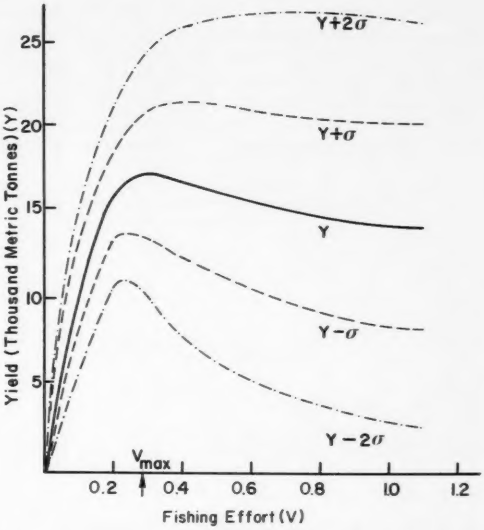

FIG. 3. Sustainable yield levels for the Georges Bank haddock fishery.

because different fisheries use different effort units (e.g. even if they both use standardized boat days as a unit of effort, their units may be based on different types of boats), a translation of v into the effort units commonly used by a particular fishery would require a catch–effort analysis of that fishery. Note also that although the curves in Fig. 1 and 3 appear to be quite different, this could be due more to the scale of the abcissa (v) than a fundamental difference in the shape of the curves.

Over the range of normalized effort values for which output was generated, yields from the anchovy fishery will on average be less variable than yields from the haddock fishery, which in turn will be on average less variable than yields from the flounder fishery (Fig. 4). Note that all three curves in Fig. 4 have a positive slope in the neighborhood of $v/v_{\max} = 1$ so that variability increases in all three fisheries as effort increases in the neighborhood of $v_{\max}$. Hence if management seeks to achieve $v_{\max}$, which it is impossible to do exactly, then it is better to underestimate $v_{\max}$ slightly and reduce yield variability than to overestimate $v_{\max}$ and increase yield variability. This is especially true for the flounder fishery which exhibits a rapid rise in variability coupled with a dramatic decline in yield (as depicted in Fig. 2) for effort levels beyond $v_{\max}$. The vulnerability of the flounder fishery to overexploitation is a consequence of poor recruitment at low stock levels (see Table 2) whereas the more robust nature of the anchovy and haddock yield curves, as depicted in Fig. 1 and 3, respectively, is a consequence of relatively stronger recruitment at low stock levels (see Table 2).

Interestingly, the curve in Fig. 4 depicting the coefficient of variation of yield for the haddock fishery has a minimum around $v/v_{\max} = 3/4$, so that implementing v somewhere in the middle of the range $[3/4v_{\max}, v_{\max}]$ represents a compromise solution in this fishery to satisfying the conflicting objectives of maximizing yield and minimizing variability.

Conclusion

The model presented in this paper provides an aid in the analysis and management of fisheries that exhibit considerable recruitment variability. It has the potential to be further developed and profitably applied to an in depth analysis of selected fisheries. In our examples we only scratched the surface of the ways in which the model can be applied to fisheries management. For example, the model can be used to study trended fisheries by changing the stock–recruitment probability transition matrices in appropriate ways and comparing the resulting yield–effort curves. The model can also be used to study the transient behavior of fisheries in addition to the sustainable yield type analysis presented above. Furthermore, it can be incorporated as part of a decision-making process, e.g. via dynamic programming, to answer specific

TABLE. 3. Results associated with the maximum sustainable yield.

Stock	Maximum mean sustainable yield $Y_{\max}$ (thousand metric tonnes)	Corresponding standard deviation $\sigma_{\max}$ (thousand metric tonnes)	Corresponding coefficient of variation $\sigma_{\max} / Y_{\max}$	Corresponding effort $V_{\max}$
Anchovy	129	19	0.15	1.8
Flounder	16.5	4.5	0.28	0.9
Haddock	17.0	3.8	0.22	0.3

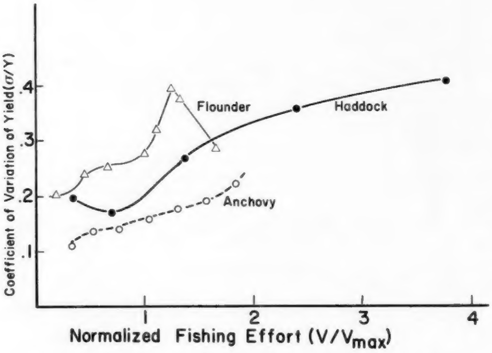

FIG. 4. Coefficient of variation of yield versus fishing effort as a fraction of the level of effort at which the maximum expected sustainable yield is obtained.

management questions. Also certain scenarios can be studied as, for example, how to take advantage of or compensate for particularly strong or weak cohorts as they pass through the fishery. Finally, the probabilities that the model generates can be used in some type of risk analysis, especially with regard to estimating the probability that the yield or adult stock will fall below an assigned critical or target level.

The model does have a drawback which may apply to certain fisheries. This is that the time required to complete a simulation run rises exponentially as the number of age-classes (n) is increased. Using the computer model we developed, we found that only simulations runs where $n \leq 6$ were practical in terms of run time and computer costs. We believe, however, that some improvements can be made to our program to reduce computational time by taking advantage of elements in the age-class and stock probability vectors that are zero or are close to zero.

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Appendix

The elements $p_{ij}(k)$ of the recruit probability vector $\mathbf{P}_i(k)$ are defined as

$$\text{(A1)}\quad p_{ij}(k) = \text{prob}\left\{x_i(k)\epsilon\left[\left(\frac{j-1}{m}\right)\hat{x}_i(k),\ \left(\frac{j}{m}\right)\hat{x}_i(k)\right]\right\},$$

$$j = 1,\ldots,m,\ i = 1,\ldots,n,\ \text{and}\ k = 0,\ 1,\ 2,\ldots.$$

The elements $p_{sj}(k+t_s)$ of the stock probability vector $\mathbf{P}_s(k)$ are similarly defined as

$$\text{(A2)}\quad p_{sj}(k+t_s) = \text{prob}\left\{s(k+t_s)\epsilon\left[\left(\frac{j-1}{m_s}\right)\hat{s},\ \left(\frac{j}{m_s}\right)\hat{s}\right]\right\},$$

$$j = 1,\ldots,m_s\ \text{and}\ k = 0,\ 1,\ 2\ldots.$$

Whenever $x_i(k)$ or $s(k+t_s)$ are considered as belonging to a certain interval, they will be given a nominal value at the midpoint of that interval. For example,

$$\text{(A3)}\quad x_i(k)\epsilon\left[\left(\frac{j-1}{m}\right)\hat{x}_i(k),\ \left(\frac{j}{m}\right)\hat{x}_i(k)\right]\Longleftrightarrow x_i(k) = \left(\frac{j-0.5}{m}\right)\hat{x}_i(k).$$

From Equation (A1) it follows directly that

$$\text{(A4)}\quad \mathbf{P}_{i+1}(k+1) = \mathbf{P}_i(k) \qquad i = 1,\ldots,n-2.$$

From Equation (A3), however, it follows that both $\mathbf{P}_{n-1}(k)$ and $\mathbf{P}_n(k)$ are required to generate $\mathbf{P}_n(k+1)$. The procedure for doing this is rather simple if the assumption can be made that $\mathbf{P}_{n-1}(k)$ and $\mathbf{P}_n(k)$ are mutually independent. From Equation (A4), this is equivalent to assuming that the distributions $\mathbf{P}_1(l)$, $l = 0,\ldots,k-n+2$ are all independent. This is clearly not the case in general, as the distribution of new recruits is dependent on the distribution of the parent stock which is directly derived from previous distributions of new recruits. The assumption appears to be reasonable, however, if there is sufficient variability in the stock–recruitment dynamics as, for example, in fisheries where recruit levels are more closely correlated with environmental factors than with parent stock (Grosslein and Hennemuth 1973; Sissenwine 1974, 1977). Under the assumption of mutual independence it follows that

$$\text{(A5)}\quad p_{nj}(k+1) = \sum_{(j_{n-1},j_n)\epsilon I_j(k)} (p_{n-1_{j_{n-1}}}(k)p_{nj_n}(k))$$

$$j = 1,\ldots,m$$

where

$$\text{(A6)}\quad I_j(k) = \left\{(j_{n-1},j_n)\middle| j_i = 1,\ldots,m,\ i = n-1,\ n,\ \text{and}\right.$$

$$\left(\frac{j_{n-1}-0.5}{m}\right)\mathrm{e}^{-(\alpha_{n-1}+q_{n-1}v_k f)}\hat{x}_{n-1}(k)$$

$$\left.+\left(\frac{j_n-0.5}{m}\right)\mathrm{e}^{-(\alpha_n+q_n v_k f)}x_n(k)\epsilon\left[\left(\frac{j-1}{m}\right)\hat{x}_n(k),\ \left(\frac{j}{m}\right)\hat{x}_n(k)\right]\right\}.$$

Under the same mutual independence assumption it follows from Equation (A2) that the distribution of the parent stock biomass is evaluated by multiplying those probabilities which result in the parent stock biomass belonging to the biomass subdivision under consideration, i.e.

$$\text{(A7)}\quad p_{sj}(k+t_s) = \sum_{(j_1,\ldots,j_n)\epsilon I_j^s(k)} p_{1j_1}(k)p_{2j_2}(k)\ldots p_{nj_n}(k)$$

$$j = 1,\ldots,m_s$$

where

$$\text{(A8)}\quad I_j^s(k) = \left\{(j_1,\ldots,j_n)\middle| j_i = 1,\ldots,m_s,\ i = 1,\ldots,n,\ \text{and}\right.$$

$$\left.\sum_{i=1}^{n} c_i w_i(k+t_s)\left(\frac{j_i-0.5}{m_s}\right)\mathrm{e}^{-(\alpha_i+q_i v_k)t_s}\hat{x}_i(k)\epsilon\left[\left(\frac{j-1}{m_s}\right)\hat{s},\ \left(\frac{j}{m_s}\right)\hat{s}\right]\right\}.$$

The new recruitment distribution at time $k+1$, i.e. $\mathbf{P}_1(k+1)$, is then generated from the parent stock distribution that was present in the fishery at time $k-r+t_s$ (recall that r is the age at which an individual is recruited to the fishery) using the stock–recruitment transition probability matrix $\mathbf{T}$, i.e.

$$\text{(A9)}\quad \mathbf{P}_1(k+1) = \mathbf{T}\mathbf{P}_s(k-r+t_s).$$

Di-*n*-hexyl Phthalate (DHP), a Newly Identified Contaminant in Atlantic Herring (*Clupea harengus harengus*) and Atlantic Mackerel (*Scomber scombrus*)

CHARLES J. MUSIAL, JOHN F. UTHE, GARY R. SIROTA, AND B. GARTH BURNS

Fisheries and Environmental Sciences, Department of Fisheries and Oceans, Halifax Laboratory, Halifax, N.S. B3J 2S7

MICHAEL W. GILGAN

Fish Inspection Branch, Department of Fisheries and Oceans, 1721 Lower Water Street, Halifax, N.S.

VLADIMIR ZITKO

Fisheries and Environmental Sciences, Department of Fisheries and Oceans, Biological Station,
St. Andrews, N.B. E0G 2X0

AND R. ALEXANDER MATHESON

Department of the Environment, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

MUSIAL, C. J., J. F. UTHE, G. R. SIROTA, B. G. BURNS, M. W. GILGAN, V. ZITKO, AND R. A. MATHESON. 1981. Di-*n*-hexyl phthalate (DHP), a newly identified contaminant in Atlantic herring (*Clupea harengus harengus*) and Atlantic mackerel (*Scomber scombrus*). Can. J. Fish. Aquat. Sci. 38: 856–859.

We describe the isolation and identification of di-*n*-hexyl phthalate, a new marine pollutant found in Atlantic herring (*Clupea harengus harengus*) and Atlantic mackerel (*Scomber scombrus*). This compound was isolated from extracted lipids by a combination of gel permeation, high performance liquid and column chromatography, and selective precipitation of impurities. Conclusive proof of its structure was obtained from gas chromatographic–mass spectrometric analysis and proton nuclear magnetic resonance spectroscopy. The concentrations of di-*n*-hexyl phthalate (17–27 μg/g wet weight), which is not a major industrial phthalic acid ester, far exceeded those of dibutyl and di-2-ethylhexyl phthalates, the major industrial phthalic acid esters. Thus the possibility of selective bioaccumulation of DHP in herring and mackerel was indicated. This finding is important in view of the paucity of toxicological information concerning this compound.

Key words: phthalic acid ester, di-*n*-hexyl phthalate (DHP), analysis, mackerel, herring, marine fish, pollution

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Nous décrivons l'isolement et l'identification du phtalate de di-*n*-hexyle, nouveau polluant marin décelé dans le hareng de l'Atlantique (*Clupea harengus harengus*) et le maquereau de l'Atlantique (*Scomber scombrus*). Ce composé a été isolé d'un extrait de lipides par chromatographie sur gel, chromatographie à haute performance en phase liquide et sur colonne et précipitation sélective des impuretés. La preuve concluante de sa structure a été obtenue par chromatographie en phase gazeuse couplé à la spectrométrie de masse et par spectroscopie de résonnance magnétique nucléaire du proton. Les concentrations de ce phtalate (de 17 à 27 μg/g de poids frais), qui n'est pas un phtalate industriel important, dépasse de loin celles des phtalates de dibutyle et de diéthyl-2 hexyle, les principaux phtalates industriels, Ainsi, la possibilité qu'il y ait bioaccumulation sélective du PDH dans le hareng

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et le maquereau est réelle. Cette découverte est importante, compte tenu du peu de renseignements toxicologiques qu'on possède sur ce composé.

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PHTHALIC acid esters (PAEs) are among the most widespread anthropogenic contaminants in the biosphere occurring, for example, in fish (Burns et al. 1981; Persson et al. 1978; Peakall 1975; Zitko 1973; Mayer et al. 1972), tadpoles (Peakall 1975), soil and freshwater arthropods (Persson et al. 1978), and cormorants and herring gulls (Zitko 1973). Their presence has also been reported in air (Bove 1978), water and sediments (Peakall 1975; Hites and Biemann 1972), various foodstuffs (Tomita et al. 1977), and human blood, which can contain up to 30 times as much PAEs as polychlorinated biphenyls (Tomita et al. 1977). Occurrence of PAEs in other human tissues has been reported (Jaeger and Rubin 1970).

To date, the PAEs which have been reported in fish from the natural environment include di-2-ethylhexyl phthalate (DEHP), di-*n*-butyl phthalate (DBP) (Burns et al. 1981; Williams 1973; Zitko 1973), and butyl benzyl phthalate (Pfuderer et al. 1975). Dimethyl-tetrachloroterephthalate (DCPA), a widely used herbicide, has also been found in fish (Miller and Gomes 1974). There is some evidence to suggest the presence of trace amounts of diethyl phthalate (DEP) in marine fish (Burns et al. 1981).

During an investigation of PAEs in marine fish a compound was detected, the chemistry and chromatography of which indicated a heretofore unreported phthalate ester occurring in fatty marine fish such as herring (*Clupea harengus harengus*) and mackerel (*Scomber scombrus*). Since this compound was present in apparently large concentrations relative to the common PAEs as DBP and DEHP, we decided to characterize it.

Materials and methods — Separation of compound — The methodology initially used to separate the compound from fish lipid was based on gel permeation chromatography (GPC) and sulfuric acid impregnated alumina chromatography (Burns et al. 1981). However, it was impractical to use this methodology to isolate enough of the compound for proton nuclear magnetic resonance (NMR) studies. Larger quantities of DHP were obtained from fish lipid by high performance liquid chromatography (HPLC) and low temperature precipitation of interferents from solutions of the appropriate GPC fraction.

Commercial size fish were taken from the Gulf of St. Lawrence in September 1978 and kept frozen round at −30°C until analysis. Composite samples of 10 fish were prepared by partially thawing, filleting, removing the skin, and homogenizing the fillets with a Hobart silent cutter. Composite liver samples were prepared by homogenizing together the whole livers from the 10 fish with a Polytron (Brinkman Inc.). The lipids were extracted from ~ 300 g of combined herring and mackerel by a previously described procedure (Musial et al. 1979). About 500-mg aliquots of lipid were chromatographed on the GPC (Autoprep 1001, ABC Laboratories, Columbia, MO) and the phthalate fractions were combined for HPLC.

HPLC was performed on a series of two normal phase columns: (1) Porasil 60(A), 37-75 μm dry-packed to produce a column 4.7 mm ID × 920 mm L, and (2) Partisil PXS 5/25 (Whatman) 5 μm, 4.60 mm × 250 mm L. The solvents were chloroform:hexane (1:1) and chloroform:hexane (15:85) (Caledon Laboratories or Fisher Scientific), respectively. Solvent flow in both cases was 2.0 mL min^{-1}. The chromatograph was equipped with an Altex model 100 pump, a Rheodyne valve injector with a 100-μL or 1000-μL loop, a Varian Variscan detector set at 250 nm, and an LKB Ultravac fraction collector.

Lipid components remaining after these procedures, as evidenced by thin-layer chromatography, were removed by a series of precipitations from cold methanol and acetonitrile (distilled in glass, Caledon Laboratories). These were performed by first dissolving ~ 20 mg of the lipid mixture in 1 mL of methanol in a 10-mL centrifuge tube at room temperature and placing the tube in a freezer at −22°C for 10-15 min, until the precipitate collected in the conical bottom of the tube. The supernatant was withdrawn by disposable pipet and placed in a second 10-mL centrifuge tube where the procedure was repeated twice with acetonitrile as the solvent. In both of these solvents, DHP is very soluble and remained in solution, while the less soluble lipid components precipitated. It should be noted that no precautions were taken to exclude air during the workup and it is believed that air oxidation assisted in rendering the lipid components less soluble in these solvents. A final trace of fatty residue, as evidenced by a tailing solvent front on GC, was removed by freeze-out at −77°C from hexane in an ethanol/dry-ice bath (Grussendorf et al. 1970).

Analysis of compound — Gas chromatography (GC) was performed with a Hewlett−Packard 5700 chromatograph with a Ni^{63} detector and the following conditions: oven, 200°C; injection port, 250°C; detector, 350°C; gas (argon/methane 95/5, from Matheson) at 50 mL min^{-1} for 4 mm ID column and 20 mL min^{-1} for 2 mm ID. Columns: (1) 180 cm × 4 mm ID glass packed with 3% SP-2100 on 80−100 mesh Supelcoport (Supelco, Inc.); (2) Outlet end: 15 cm × 2 mm ID 3% SP-2100/3% OV-210; inlet end: 170 cm × 2 mm ID 1% SP-2100/2% SP-2401 on 100−120 mesh Supelcoport, coated separately and mechanically mixed before packing.

Proton nuclear magnetic resonance (NMR) was performed on a Varian FT-80A using $CDCl_3$ solvent and TMS internal standard in a 30°C probe. The following conditions applied: filter band width, 1000 Hz; pulse width, 47 s; data points, 8190; sweep width, 1000 Hz, acquisition time, 4.095 s; pulse delay, 6.000 s; completed transients, 25; width of plot, 810 Hz.

Results and discussion — The large unknown phthalate peak which appeared in the electron capture gas chromatogram of the GPC phthalate fraction of mackerel is shown relative to DEHP in the same sample in Fig. 1. GC−MS studies indicated that this was indeed a phthalate-based contaminant, having the characteristic base peak at *m/z* 149. However, it was not possible to elucidate the structure(s) of

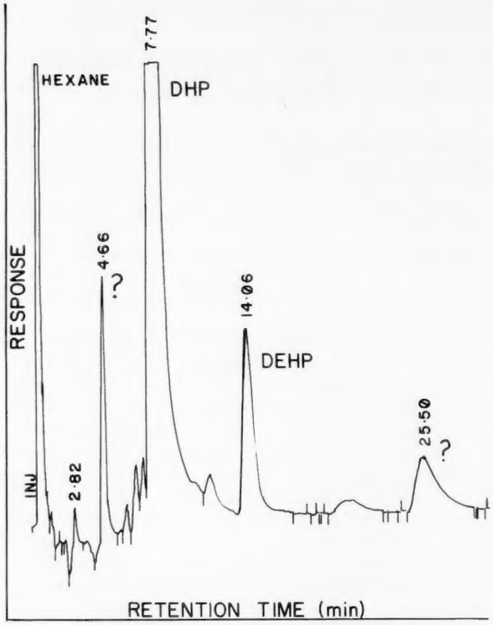

FIG. 1. Electron capture gas chromatogram of GPC phthalate ester fraction of Gulf of St. Lawrence mackerel, cleaned up on sulfuric acid impregnated alumina. Numbers above peaks indicate retention times in minutes. ? = unknown.

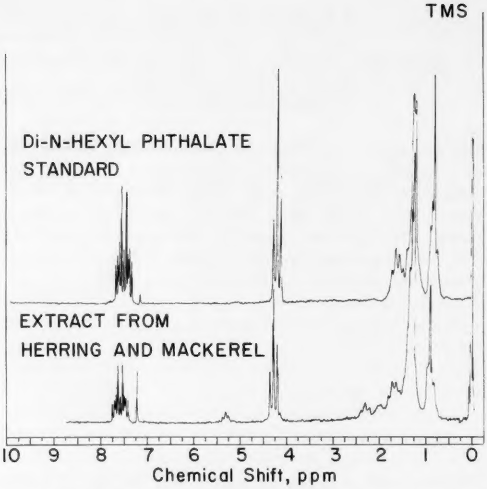

FIG. 2. NMR spectrum of phthalate ester extracted from herring and mackerel compared to that of authentic DHP. ppm (parts per million) represents the chemical shift in cycles per second divided by the oscillator frequency.

the alkyl moieties from the mass spectrum. Therefore, we decided to isolate a sufficient quantity for proton NMR.

Normal phase HPLC yielded about 20 mg of clear oily liquid which appeared to be pure by GC and normal phase HPLC.. NMR spectroscopy of this liquid showed large quantities of highly saturated compounds (possibly fatty acids) and small quantities of other esters (probably triglycerides). Thin-layer chromatography on silica gel confirmed the presence of such components. Removal of these interferents gave a few milligrams of an essentially pure compound as indicated by reverse phase HPLC and packed column and capillary GC. The retention time of the unknown was identical with that of standard DHP (Chem Service) by these three types of chromatography. However, certain phthalates coelute, for example, DEHP and di-*n*-heptyl; therefore, conclusive proof of the identity of the unknown was given by NMR spectroscopy. Very good agreement between NMR spectra of the unknown and standard DHP was demonstrated (Fig. 2). A minor impurity (probably a small amount of triglyceride) could be detected in the spectrum of the compound extracted from fish lipid.

Initial GC estimates of DHP concentrations in fish indicated that the compound was present in concentrations of 17.0 μg/g wet weight in herring and 27.2 μg/g wet weight in mackerel fillets while it was not detectable (0.010 μg/g) in plaice (*Hippoglossoides platessoides*) and redfish (*Sebastes marinus*) fillets and cod (*Gadus morhua*) liver taken from the same area of the Gulf of St. Lawrence in 1978 (Table 1).

It is interesting to note that the concentrations of DHP in herring and mackerel fillets were much greater than those of DEHP in the same fish (Table 1). This was entirely unexpected in view of the low commercial production volume of DHP as compared with DEHP and DBP. Actual production volume of DHP was not available in the literature but it has been reported that DEHP, DBP, DEP, diisodecyl, diisooctyl, and *n*-octyl *n*-decyl phthalates together account for 70% of the total production of PAEs (Fishbein and Albro 1972). The lack of any information about DHP even in most technical and plasticizer manufacturers' literature indicates that the amount of industrially produced DHP is probably only a small percentage of that of DEHP. One of the few references to DHP in the scientific literature is that of its detection in lipid extracts of plasma from transfusion packs (Marcel and Noel 1970). However, this finding was refuted by Gesler and Kartinos (1970), who placed evidence to show that this was a case of mistaken identity (the compound actually being DEHP). No reference to DHP occurring in any fish could be found.

The source of the relatively large concentrations of DHP in herring and mackerel is unknown. Diet is a distinct possi-

TABLE 1. Phthalate ester concentrations in commercial fish (pooled samples of 10 individuals each).

	DHP, μg/g wet wt.	[a]DEHP, μg/g wet wt.
Herring (fillets)	17.0	4.71
Mackerel (fillets)	27.2	6.50
Cod (liver)	<0.010	5.19
Plaice (fillets)	<0.010	<0.010
Redfish (fillets)	<0.010	<0.010

[a]Data from Burns et al. 1981.

bility. Herring and mackerel are both pelagic species feeding mainly on plankton. DHP could not be found in plaice and redfish fillets and the liver of small cod. These three are benthic species, feeding mainly on bottom-dwelling organisms such as mollusks and crustacea. It is feasible that DHP, especially if recently introduced into the marine environment, might be confined to species in the pelagic food web. Water is a possible source but in this case one would expect the contaminant to be present in all species tested. Lastly, a metabolic process in mackerel and herring such as de-ethylation of DEHP could give rise to DHP from DEHP. However, this would be in disagreement with the findings of others, which indicate that hydrolysis of phthalate diester to the monoester and free acid is common since the usual PAE metabolities are conjugated and unconjugated monoesters and phthalic acid (Mayer 1976; Melancon and Lech 1976; Stalling et al. 1973). These studies were performed on freshwater fish of the Cyprinidae (minnows, carp), Salmonidae (trout), and Ictaluridae (catfish) families. To our knowledge, no studies have been done on the Clupeidae (herring, shad, menhaden) and Scombridae (mackerel, tuna) families.

The ultimate source of DHP in the marine environment (assuming that it was introduced as DHP) is a matter of speculation. It is possible that DHP is a contaminant in commercial DEHP. Brownlee and Strachan (1977) reported the presence of di-*n*-octyl phthalate in the receiving waters of a kraft pulp and paper mill on Nipigon Bay, Lake Superior; it is possible that DHP comes from a similar source

Further work is required to investigate the source and extent of contamination by DHP of the aquatic biota.

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Measuring Nutrient Spiralling in Streams[1]

J. DENIS NEWBOLD, JERRY W. ELWOOD, ROBERT V. O'NEILL, AND WEBSTER VAN WINKLE

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA

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Nutrient cycling in streams involves some downstream transport before the cycle is completed. Thus, the path traveled by a nutrient atom in passing through the cycle can be visualized as a spiral. As an index of the spiralling process, we introduce spiralling length, defined as the average distance associated with one complete cycle of a nutrient atom. This index provides a measure of the utilization of nutrients relative to the available supply from upstream. Using ^{32}P as a tracer, we estimated a spiralling length of 193 m for phosphorus in a small woodland stream.

Key words: downstream transport, nutrient cycling, phosphorus, spiralling, stream

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Le cycle des éléments nutritifs dans les cours d'eau comporte un certain transport en aval avant que le cycle soit complété. On peut donc concevoir comme une spirale le chemin parcouru par un atome de nutriment durant ce cycle. Comme indice du processus, nous introduisons la longueur du trajet en spirale, définie comme la distance moyenne associée avec un cycle complet d'un atome de nutriment. Cet indice est une mesure de l'utilisation des éléments nutritifs par rapport à la quantité disponible en amont. Avec le ^{32}P comme marqueur, nous estimons que dans un petit cours d'eau traversant un boisé, la longueur du trajet en spirale du phosphore est de 193 m.

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NUTRIENTS, such as nitrogen and phosphorus, are critical to the maintenance of ecosystem function. Their cycling affects the ability of an ecosystem to withstand and recover from perturbations (Webster et al. 1975; Bormann and Likens 1979). Webster (1975; Webster and Patten 1979) pointed out that nutrients in a stream do not cycle in place, but are displaced downstream as they complete a cycle. He described this coupling of transport and cycling as "spiralling" and suggested that the ability of a stream to utilize nutrients is associated with the "tightness" of the spirals. Other workers have referred to the concept of spiralling (Wallace et al. 1977; Naiman and Sedell 1979; Meyer 1979; Benke and Wallace 1980), or recognized the importance of spatially dependent reutilization of nutrients (Ball and Hooper 1963; Short and Maslin 1977) and organic matter (Fisher 1977). In this paper we introduce an index of nutrient spiralling and report a field measurement of this index.

To develop the index, we assume that a nutrient atom "cycles" through three generalized compartments (water, particulates, and consumers) of a stream ecosystem (Fig. 1). Each compartment is continuously transported downstream, so that the path of the nutrient describes an imaginary spiral. We can quantify this path in terms of spiralling length (S) (Fig. 1a), defined as the average distance required to complete one loop of the spiral. The three components of spiralling length are the average distance a nutrient atom moves in the water compartment (S_W), in the particulate compartment (S_P), and in the consumer compartment (S_C). The shorter the spiralling length, the more efficiently the nutrient is utilized, i.e. the more times a nutrient atom is recycled within a given reach of stream.

Methods — The experiment involved releasing 370 MBq each of ^{32}P (as carrier-free $K_2\,^{32}PO_4$) and ^{3}H (as tritiated water) in July 1978 in the West Fork of Walker Branch, a small woodland stream on the Department of Energy Reservation (Curlin and Nelson 1968) in Oak Ridge, Tennessee, USA. Water samples for radioassay were taken from 11 stations at 3- or 6-min intervals as the 30-min release slug passed through the 120-m reach. Total passage of ^{32}P past a station was estimated by integrating the concentration vs. time curve and applying dilution corrections obtained from the ^{3}H data. Radioactivity in sediments, seston, and biota was monitored for several weeks thereafter.

Results and discussion — The first component of spiralling length, S_W, was estimated from uptake of ^{32}P from water to particulates. As Ball and Hooper (1963) observed, uptake of

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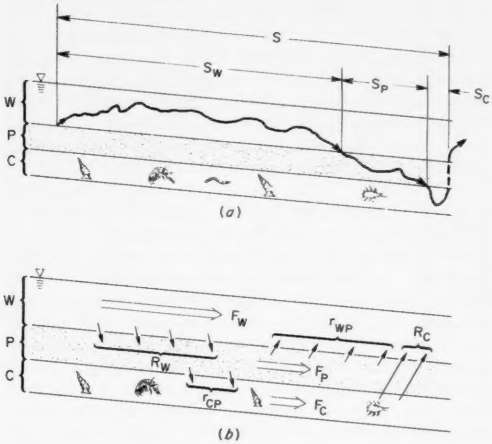

FIG. 1. Nutrient spiralling in a stream ecosystem. (a) The three components of spiralling length, S. The nutrient atom travels an average longitudinal distance, S_W, dissolved in the water compartment, W, plus an average distance, S_P, in the particulate compartment, P, plus an average distance, S_C, in the invertebrate consumer compartment, C, before returning to the water. (b) Downstream nutrient fluxes, F (g · s^{-1}), and exchange fluxes, R (g · s^{-1} · m^{-1}), of nutrients between compartments. The total release from the particulate compartment, R_P, consists of the sum of the release to the water, r_{WP}, and the uptake by consumers, r_{CP}.

^{32}P at each point downstream is proportional to the ^{32}P remaining in the water at that point. Therefore, the proportion $A(x)$ of ^{32}P remaining in the water x metres downstream from the point of release decreases exponentially with distance downstream; i.e. $A(x) = \exp(-k_W x)$, where k_W is the distance rate constant (m^{-1}). Then, the average distance traveled downstream by a ^{32}P atom, calculated as the cumulative distance traveled by all of the ^{32}P atoms divided by the number of ^{32}P atoms initially present, is given by $S_W = 1/k_W$ (Overman and Clark 1960, p. 288).

Uptake of ^{32}P in Walker Branch conformed to the exponential model (Fig. 2), giving an estimate for k_W of 0.0060 m^{-1}, or a value of 167 m for S_W. If significant release (i.e. cycling) of ^{32}P from particulates occurred during the passage of the tracer slug (which approached 90-min duration at 120 m), S_W would be overestimated by this approach. As the tracers left the reach, however, water concentrations of both ^{3}H and ^{32}P dropped simultaneously to below detection limits, indicating that release rates of ^{32}P were too small to interfere with the uptake esitmate.

We can interpret S_W and the other two components of spiralling length in terms of the supply and utilization rates of dissolved P. We define F_i as the supply rate or downstream flux of phosphorus in compartment i; it is the amount of phosphorus passing through a given cross section of stream per unit time. We define R_i as the utilization rate or exchange flux of phosphorus from compartment i; it is the amount of phosphorus per unit time lost from compartment i per unit length of stream bottom. As the downstream flux (F_i) travels a unit length of stream bottom, a proportion of this flux, R_i/F_i, is lost from compartment i. If we trace the downstream rate of disappearance of phosphorous atoms without interference from replacement fluxes from other compartments, the rate of disappearance of the tracer over distance is a direct measure of this ratio, R_i/F_i. Thus, the distance rate constant (k_i) equals R_i/F_i, and the average distance or average number of unit lengths of stream bottom a phosphorus atom moves downstream in compartment i is given by $1/k_i = S_i = F_i/R_i$. In terms of the nutrient fluxes shown in Fig. 1b, the first component of spiralling length can be calculated as $S_W = F_W/R_W$, where F_W is the downstream flux (g · s^{-1}) of dissolved nutrient in the water, and R_W is the uptake flux per unit length of stream bottom (g · s^{-1} · m^{-1}) from water to particulates and associated microbes.

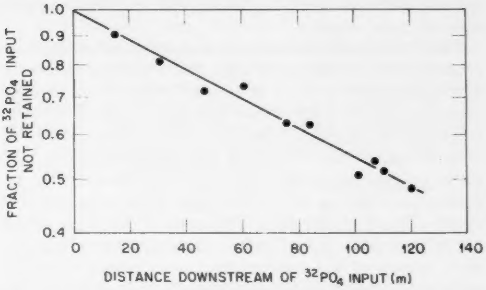

FIG. 2. Proportion of total ^{32}P input remaining in the water versus distance downstream from the point of release. Points represent time-integrated values for ^{32}P transport. Line is the function $A(x) = \exp(-k_W x)$, where $k_W = 0.0060$ m^{-1}.

The second component of spiralling length can be calculated in an analogous manner as $S_P = F_P/R_P$, where F_P (Fig. 1b) is the downstream flux (g · s^{-1}) of nutrient in the particulate phase, and R_P is the release flux per unit length of stream bottom (g · s^{-1} · m^{-1}) from the particulates. This release consists of loss to the water (r_{WP}) plus loss to consumers (r_{CP}). For a stream in steady state, release flux from particulates equals uptake flux from water to particulates (i.e. $R_P = R_W$). Therefore

$$S_P = F_P/R_W = (F_P/F_W)\,F_W/R_W = (F_P/F_W)\,S_W.$$

Thus, under these conditions, S_P can be determined from S_W if F_P/F_W is known. In July – August 1978, soluble reactive phosphorus (SRP) (Chamberlain and Shapiro 1969) averaged 4.1 μg/L and total phosphorus in suspended particulates averaged 1.9 μg/L. Laboratory equilibration experiments, however, showed that only 34% of the particulate P is exchangeable with the dissolved P pool in Walker Branch (Elwood et al. 1981). Considering only the exchangeable portion of the particulate P, we obtain $F_P/F_W = 0.16$. From the equation given above, $S_P = 0.16\ S_W = 26$ m. The soluble reactive phosphorus analysis may have overestimated the available P in the water (Rigler 1966; Stainton 1980), but the error would have produced a compensating overestimate of exchangeable pool size in particulates, because the latter estimate also incorporated a chemical determination of SRP in Walker Branch water.

Once a nutrient atom enters the consumer compartment, its expected travel distance is F_C/R_C, where F_C (Fig. 1b) is the downstream flux ($g \cdot s^{-1}$) of nutrient in the consumer compartment, and R_C is the release flux ($g \cdot s^{-1} \cdot m^{-1}$) from the consumers by excretion to the water compartment. However, only the fraction (b_C) of nutrient that passes through the consumer compartment, rather than returning directly from particulates to water, actually contributes to the total spiralling length. Therefore, the third component of spiralling length can be given by $S_C = b_C\,(F_C/R_C)$. At steady state, release flux from consumers is a constant fraction of release flux from particulates, and $R_P = R_W$. Therefore $b_C = R_C/R_P = R_C/R_W$, and S_C can be written in terms of S_W and downstream fluxes:

$$S_C = F_C/R_W = (F_C/F_W)\,S_W.$$

Phosphorus in drift, on a volume basis, was 0.002 μg/L, all of which was assumed exchangeable. Therefore, $F_C/F_W = 5 \times 10^{-4}$, and $S_C = 0.08$ m. Downstream transport in consumers contributes negligibly to the total spiralling length, $S = S_W + S_P + S_C = 193$ m.

From the relations developed above, total spiralling length can be expressed as the ratio of total downstream flux of nutrient (F_T) to R_W:

$$S = F_W/R_W + F_P/R_W + F_C/R_W = F_T/R_W.$$

The importance of this ratio is that spiralling length provides a relationship between nutrient utilization (R_W) and total nutrient supply (F_T). If nutrient utilization in a stream is to increase, S must decrease. Adaptations and functions that reduce downstream transport (e.g. holdfast devices and filter feeding) will tend to shorten the spiral. While the definition of spiralling length as the expected distance to complete one cycle does not depend on whether the stream is at steady state, both our calculations and the interpretation in terms of total nutrient fluxes apply only to the steady state. An alternate approach to the calculation of S_P and S_W that does not depend on the steady-state assumption will be considered in a future publication.

Upstream–downstream comparisons of ^{32}P loss demonstrate that spiralling occurred. Figure 3 shows that ^{32}P loss from leaf detritus (≥1 mm), which accounted for ~ 60% of ^{32}P uptake, was slower downstream (open circles) than upstream (closed circles). This indicates that some of the ^{32}P released upstream was resorbed downstream.

A simple model for transport and exchange of ^{32}P in the stream is given by:

$$\frac{\partial Q_W}{\partial t} = D\,\frac{\partial^2 Q_W}{\partial x^2} - v\,\frac{\partial Q_W}{\partial x} - \frac{v}{S_W}\,Q_W + k\,Q_P, \text{ and}$$

$$\frac{\partial Q_P}{\partial t} = \frac{v}{S_W}\,Q_W - k\,Q_p,$$

where D is longitudinal dispersion and v is water velocity (both estimated from ^{3}H tracer data), $Q_W(x,t)$ is ^{32}P concentration in water, $Q_P(x,t)$ is ^{32}P concentration in particulates, and k is the turnover of ^{32}P associated with particulates. Because S_P is small relative to S_W, the transport of particulates is neglected in this model. With S_W set at the measured value of 167 m, k was estimated by fitting the model to the 20-m data (broken line in Fig. 3). With no further fitting, the model predicted the curve shown through the data from 120 m. Other values of S_W, over the range $130 < S_W < 200$ m, produced poorer but acceptable fits. The ability of the model to predict this reutilization of ^{32}P provides a rough validation of our estimate of spiralling length. In lakes, a significant amount of regenerated phosphorus may be associated with large molecules and unavailable for immediate re-uptake (Lean 1973). Our result suggests that, in Walker Branch, the availability of regenerated P is not very different from that of orthophosphate.

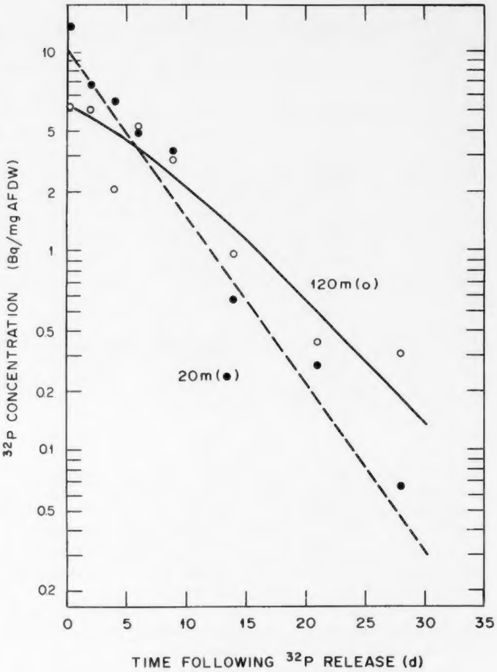

FIG. 3. Mean concentrations of ^{32}P (Bq/mg ash-free dry weight) in leaf detritus (≥1 mm) from Walker Branch at two locations downstream after a 30-min release of $^{32}PO_4$ as a function of time following the release. Broken line shows model calibration to data from 20 m; solid line is model prediction for 120 m.

The spiralling concept provides a basis for considering the role of ecosystem structure in governing nutrient utilization. Travel distance in the water (S_W) dominated the spiralling length, indicating that uptake from the water may be the primary constraint on increased nutrient utilization. The small value for the consumer component, S_C, does not imply that the role of consumers in spiralling is negligible, but it indicates that downstream drift of consumers does not significantly limit nutrient utilization. The indirect influence of consumers may be important. Feeding activities of grazers and shredders (Cummins and Klug 1979) decrease organic particle sizes, which can increase the surface area available for sorption and microbial activity (Hargrave 1977). In addition to increasing nutrient uptake from water, which would reduce S_W, consumers may also reduce particulate turnover length, S_P, by preventing buildup and sloughing of the aufwuchs community and reducing particulate flux, F_P.

Abiotic factors clearly would have a major influence on spiralling. For example, we would expect spiralling length to increase with increasing streamflow, and we would expect physical, rather than biological, explanations to dominate comparisons of spiralling length in streams of very different morphometry. An index such as uptake per unit area (R_W/stream width), which can be calculated for a field measurement of S_W and F_W, may be appropriate for purely biotic comparisons. Storms and seasonal variations can have a major influence on the phosphorus budgets of streams (Meyer and Likens 1979; Rigler 1979), and they undoubtedly affect spiralling length. Analysis of these variations in terms of their effect on spiralling may help to elucidate the relations between biotic and abiotic factors controlling nutrient utilization.

Spiralling length, then, is an index of the utilization of nutrient supplied from the surrounding watershed. The concept incorporates both reuse and retention, and it provides an approach for relating downstream fluxes to instream metabolic activity. Spiralling length is analogous to a recycling index (Finn 1976; Richey et al. 1978), but it explicitly considers the longitudinal dependence of nutrient recycling in the stream.

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Extracellular Secondary Metabolite Production by the Marine Dinoflagellate *Prorocentrum minimum* in Culture

CHARLES G. TRICK, PAUL J. HARRISON,[1] AND RAYMOND J. ANDERSEN[2]

Department of Oceanography, University of British Columbia, Vancouver, B.C. V6T 1W5

TRICK, C. G., P. J. HARRISON, AND R. J. ANDERSEN. 1981. Extracellular secondary metabolite production by the marine dinoflagellate *Prorocentrum minimum* in culture. Can. J. Fish. Aquat. Sci. 38: 864–867.

Marine dinoflagellates produce extracellular secondary metabolites that may play a role in the ecology of the producing species. The concentration of one such external metabolite, 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione, produced by the marine dinoflagellate *Prorocentrum minimum*, was determined quantitatively during exponential growth and during senescence in phosphate-starved batch cultures. The pattern of production is similar to the production of many bacterial toxins. There was little production of the β-diketone during the exponential growth period and highest production occurred within 1 wk after cessation of cell division. About 50% of the total β-diketone produced was excreted on a single day, 6 d after phosphate became limiting to growth. Cell lysis or photodestruction of carotenoids do not appear to be the source of this compound.

Key words: antibiotic, dinoflagellate, organic excretion, *Prorocentrum minimum*, secondary metabolite, phosphate starvation

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Les dinoflagellés marins produisent des métabolites secondaires extracellulaires qui peuvent jouer un rôle dans l'écologie des espèces qui les produisent. L'un de ces métabolites externes, la (triméthyl-2,6,6 hydroxy-4 cyclohexényl)-1 butanedione-1,3, produite par *Prorocentrum minimum*, a été dosé au cours de la croissance exponentielle et du vieillissement de lots de culture carencés en phosphates. Sa production a une allure similaire à celle de beaucoup de toxines bactériennes. La production de la β-dicétone a été très faible au cours de la croissance exponentielle et elle a été maximale moins d'une semaine après l'arrêt des divisions cellulaires. Près de la moitié de toute la β-dicétone produite a été excrétée au cours d'une seule journée, 6 jours après que les phosphates furent devenus un facteur limitant de la croissance. La lyse cellulaire ou la photolyse des caroténoïdes ne semble pas être à l'origine de ce composé.

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MARINE phytoplankton excrete a wide variety of organic compounds; however, the magnitude and the conditions causing excretion are still matters of controversy (Smith and Wiebe 1976; Sharp 1977; Fogg 1977; Mague et al. 1980). Most of the reported excreted material to date has been simple compounds such as organic acids, amino acids, and carbohydrates (Hellebust 1974). Excretion of large molecular weight compounds has been reported and the inhibitory or stimulatory effects observed during species interactions experiments have been attributed to these compounds (Uchida 1977; Pintner and Altmeyer 1979).

Very few extracellular secondary metabolites from marine phytoplankton have been chemically characterized. Most of the compounds investigated prior to our work have been intracellular toxins and sterols from dinoflagellates (Shimizu et al. 1976; Finer et al. 1978; Alam et al. 1979; Schantz 1979; Withers et al. 1979) and toxins, alkaloids, and novel lipids from marine cyanobacteria (Moore 1977; Mynderse and Moore 1978; Cardellina et al. 1978, 1979; Cardellina and Moore 1980). There is increasing interest in interactions between species in the field and in culture. Extracellular metabolites are thought to play an important role in many of the observed competitive interactions between species (Pratt 1966) and the control of production of individual excreted compounds, by environmental and physical factors, has not been examined previously.

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[1] Also Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1W5.

[2] Also Department of Chemistry, University of British Columbia, Vancouver, B.C. V6T 1W5.

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Recent work has provided conflicting results on the ability of *Prorocentrum* species to produce compounds inhibitory to other algal species (Elbrächter 1976; Uchida 1977; Iwasaki 1979; Kayser 1979). We have been able to isolate an extracellular antibiotic from filtrates of *Prorocentrum* culture and we have shown its chemical structure to be the norcarotenoid, 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione(Fig. 1; Andersen et al. 1980). Compounds of this type have also been shown to be produced by *Cyanidium* (Rhodophyceae) in mass culture (Jüttner 1979). As part of a larger investigation to evaluate the bioactive nature of extracellular metabolites from marine dinoflagellates, we examined the kinetics of production of this external secondary metabolite by *Prorocentrum minimum* grown in the laboratory during exponential growth and during defined conditions of phosphate starvation.

Materials and Methods — *Prorocentrum minimum* was originally isolated from English Bay, B.C., Canada, and has been maintained in the Northeast Pacific Culture Collection (NEPCC No. 96) since 1971. An axenic isolate was grown in sterile enriched artificial seawater, modified by the replacement of Na glycerophosphate with Na_2HPO_4 and $Fe(NH_4)_2SO_4$ with $FeCl_3$ (Harrison et al. 1980). The amount of phosphate in the enrichment solution was reduced to 6 μmol/L (N:P = 27:1) to ensure that cultures became phosphate-starved during senescence. Continuous light was provided by daylight fluorescent bulbs at an irradiance of 160 $\mu E \cdot m^{-2} \cdot s^{-1}$ and a temperature of 18°C. Cells were grown axenically in 6-L batch cultures with constant stirring (60 or 120 rpm).

Cultures were tested frequently to determine if they remained axenic throughout the experiment by plating on an organic-containing sterility medium. Disappearance of phosphate from the medium was monitored to ensure phosphate deficiency. Cultures were examined daily and cells were counted using a Palmer–Maloney chamber.

Several identical cultures were set up and each culture was completely harvested at different stages of growth. Production during the growth cycle is a compilation of three distinct experimental repetitions. Cells were removed from the medium by passing the 6-L cultures through a continuous centrifuge (Sharples, type TL). The cell-free medium was membrane-filtered (0.45 μm) and adjusted to pH 2.0. A dissolved organic fraction was collected by passing the acidified filtrate through a 25 × 2 cm column of precleaned (Soxhlet extraction with methanol, 72 h) XAD-2 resin (Mallinckrodt). Remaining salts were removed by washing the column with 200–300 mL deionized, distilled water. The collected organic fraction was eluted with 150 mL methanol.

The methanol extract was concentrated by rotary evaporation at 35°C in vacuo and the residue was partitioned between $CHCl_3$ and H_2O. Evaporation of the $CHCl_3$ in vacuo at room temperature provided the crude filtrate extract.

Pure β-diketone (Fig. 1) was used as an analytical standard (Andersen et al. 1980). All crude extracts were quantitatively determined via HPLC equipped with a silica gel column (LiChrosorb S160) using isocratic separation (2 mL/min; hexane:isopropanol, 83:17) and UV detection (287 nm).

To determine the efficiency of the extraction procedure, we introduced a known amount of standard compound into two replicate flasks with 6 L of seawater medium and, after extraction, assayed by HPLC and by absorbance in methanol (287 nm).

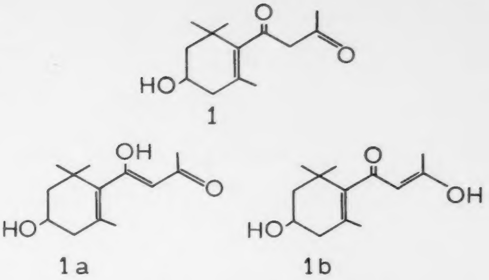

FIG. 1. Structure of the extracellular metabolite [1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione] from the marine dinoflagellate, *Prorocentrum minimum*. Spectral data show that the β-diketone exists exclusively as one of the enol tautomers Ia or Ib (Andersen et al. 1980).

To estimate the importance of the β-diketone in relation to the total organic excretion by the culture, we measured excretion using two different methods. Dissolved organic carbon (DOC) in the culture filtrate was determined by wet oxidation with potassium persulfate, with correction for reagent and medium blanks. Rates of excretion were estimated using the $H^{14}CO_3^-$ technique of Mague et al. (1980). Radioactivity was measured in a Nuclear-Chicago Unilux 111 liquid scintillation counter. Cells were incubated for 4 h under experimental conditions.

Results and Discussion — Only two compounds were found in the extract of the culture filtrate from *P. minimum*. The main compound was identified to be the β-diketone (Fig. 1; Andersen et al. 1980), while the second compound was only present in minor concentrations and was not identified or examined further.

The production of the β-diketone during culture growth is shown in Fig. 2. Little β-diketone was produced during exponential growth. Cells became phosphate-limited after 7 d of growth and ~50% of the compound was produced in a single 'pulse' 6 d later. Decreasing amounts were produced during the later stages of senescence under phosphate starvation.

Based on rates of excretion determined using the $H^{14}CO_3^-$ technique, the β-diketone comprises about 2.8% of the total amount of carbon produced on day 13 (the period of maximum β-diketone production). The average production over the entire period of β-diketone production (day 11 through day 19) is much lower, with the β-diketone accounting for an average of 0.4% of the carbon released per day. This is similar to calculations based on direct DOC determinations where the β-diketone comprises 0.6% of the total DOC at the end of the experimental period.

The observed pattern of production and the relatively high light intensities utilized prompt the concern that the occurrence of this compound in the medium is a result of either cell lysis or the photochemical oxidation of carotenoids. It does

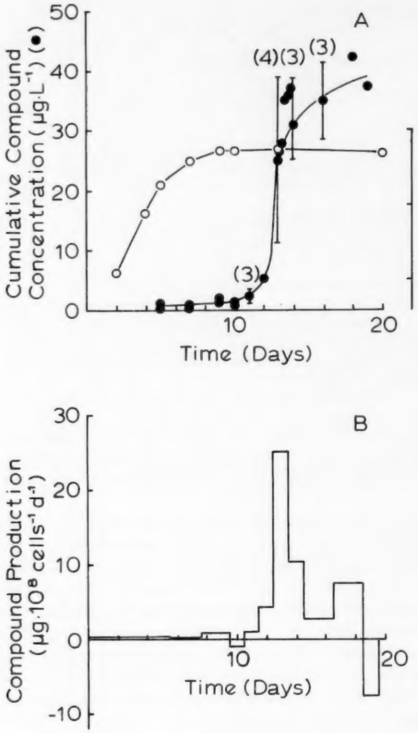

FIG. 2. *Prorocentrum minimum*. (A) Changes in 1-(2,6,6-trimethyl-4-hydroxycyclohexenyl)-1,3-butanedione concentration and cell numbers during exponential growth and during senescence in phosphate-starved batch cultures. Values are single experiments unless noted. Replicate compound concentration values are mean ±SE, where *n* is noted in parentheses. (B) Rates of compound production. Extraction efficiency of the β-diketone was 78%; concentration values have been corrected accordingly.

not seem likely that the compound is released as a result of cell lysis since loss of cells based on cell count was only noted 18 d after most of the β-diketone had been released. In addition, we have never found intracellular β-diketone in this *Prorocentrum* isolate. Furthermore, our HPLC technique indicated the production of only one main and one minor compound in this extracellular fraction throughout the entire growth period except for day 30, where a variety of compounds were collected.

A second consideration is whether this compound is the result of in vitro photooxidation of carotenoids. The structure of the β-diketone suggests a possible carotenoid origin, and preliminary data indicate the compound is produced only at saturating light intensities. It has been shown that nor-carotenoids of similar carbon skeleton are produced by photooxygenation of carotenoids in vitro (Isoe et al. 1969; Isoe et al. 1972). We believe, however, that the β-diketone is a product of an enzyme-controlled metabolism, perhaps involving

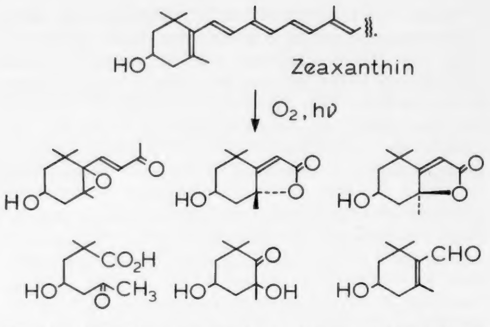

FIG. 3. Zeaxanthin and products of photooxygenation (adapted from Isoe et al. 1972).

a photobiochemical step, rather than a nonspecific photooxidation for the following reasons: (a) *P. minimum* produces only one compound of this type. If nonenzymatic chemical breakdown of carotenoids is occurring, one would expect all carotenoids to undergo degradation and several compounds would be produced in direct proportion to the carotenoid composition. The precise carotenoid composition of *P. minimum* is not known; however, members of this genus have been reported to contain two major xanthophylls (peridinin and diadinoxanthin), with β-carotene constituting less than 10% of the total carotenoid content (Johansen et al. 1974). Zeaxanthin and violaxanthin, two likely precursors of the β-diketone, have not been reported in members of the Dinophyceae (Goodwin 1979), while on a structural basis peridinin does not appear to be a likely precursor. However, we envisage that the β-diketone could have originated from diadinoxanthin by hydration of the acetylene bond followed by oxidative rupture of the carotenoid chain at C_8–C_9; (b) Isoe et al. (1972) have shown that the in vitro photooxygenation of zeaxanthin produces a complex mixture of products (Fig. 3) while our isolation of a single nor-carotenoid argues against such nonspecific photooxidations; and (c) the low rate of production during the initial period of phosphate starvation, followed by a 'pulse' of excretion during later stages, suggests some form of biological control.

While we believe this to be the first report on the pattern of production of an extracellular secondary metabolite from phytoplankton, this pattern is not unique among microorganisms. The production pattern is very similar to that observed for many bacterial toxins, where there is no production during exponential growth, low production as cells undergo adjustment from exponential growth to the stationary phase, high production during the early stationary period, and a rapid cessation of production after a short period of time (Weinberg 1970). Usually the length of production period is between one half to two times the length of the exponential growth period in bacterial cultures (Weinberg 1970). In this study, 50% of the material is produced in a single pulse suggesting a readily available precursor pool or a rapid exhaustion of available precursors.

In conclusion, we have established the first pattern of production of an identifiable extracellular secondary metabolite produced by a marine dinoflagellate. Since our data show production only under nutrient-starved conditions and a low yield of compound when compared to total organic excretion determined by other techniques, we must conclude that under open ocean conditions, the production of this compound would have negligible influence on organic carbon cycling. However, we cannot ignore the important influence this compound may exert on species succession, especially under bloom conditions in which *Prorocentrum* has occasionally been found to be abundant (Nakazima 1968; Kat 1979).

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BOOK REVIEWS/COMPTES RENDUS

Book review editor:
David P. Scott
Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

The Fishes of Illinois. *Philip W. Smith. The University of Illinois Press, Champaign, IL. 1979. xxix + 314 p. $20.00 U.S.*

In 1961, Smith wrote a definitive treatise on *The Amphibians and Reptiles of Illinois* that presumably fulfilled his goals in a proper faunal study. He then turned his attention to the production of a companion work on Illinois fishes. In this task, he worked in the shadow of Forbes and Richardson's (1908) classic monograph on Illinois fishes that for nearly a half century was unequaled in North America as a regional ichthyology. Based on 1545 collections from all sections of the state (a notable undertaking when travel was largely by boat and horse-drawn vehicles), the study included an atlas which introduced the practice of showing spot-distribution maps; most of the 150 then recognized native species were mapped. A thorough hydrographic account of Illinois, detailed descriptions, and beautiful color illustrations of many fishes and much ecological and distributional information were provided. Smith's plans were further modified by the appearance of taxonomic revisions and regional ichthyologies of nearby areas that adequately covered some topics and approaches.

In the new *Fishes of Illinois*, Smith has elected, insofar as possible, to complement Forbes and Richardson. He has produced an authoritative guide to identification of Illinois fishes with practical keys and skeleton synonymies and diagnoses, but has avoided extended descriptions. Variational data for most species are succinct, with references to other accounts. Ecological information, life history data, and habitat requirements, although reliable, make no attempt at being encyclopedic. For each species there is a distribution map and for most a large (about 14–16 cm) drawing. Because of a large fauna and central location, *The Fishes of Illinois* will serve as a useful tool in Illinois, for students and fishermen in adjacent areas, as well as for ichthyologists everywhere. Illinois distributions, present and former, are adequately shown by the maps that accompany each species. There are no general distribution maps, and text descriptions of ranges outside Illinois are infrequent.

Prefatory material in the volume is condensed, occupying fewer than 20 pages, and includes a history of Illinois ichthyology, an all-too-brief résumé of cultural and ecological change, maps showing counties, principal rivers, reservoirs and early and recent collection sites, a summary of exotic, extirpated, decimated, rare and/or restricted species, an organizational guide, glossary, and a readily workable key to the 28 included families.

Accounts of species, together with brief general statements about families and genera, occupy 299 pages, and 14 pages are devoted to references and index. The Illinois fish fauna consists of 199 species (a late discovery recorded on page 211 brings the number to an even 200) of which 13 are either introduced or are intruders that have utilized man-made waterways. Nine and perhaps 12 species have been extirpated in the recent past, and several others barely survive. The substantial increase in the known faunal list between 1908 (150 native species) and 1979 results in part from recent discoveries of forms that narrowly enter the state or are rare. However, this increase stems largely from discriminating taxonomic work of this century that has shown former "species" to be complexes of two or more forms. For example, Forbes and Richardson admitted two species of *Hybognathus*, of which one has been transferred to another genus, but five species are reported by Smith. The number of species of *Notropis* has increased from 17 to 28 even though two have been removed.

Illinois fish books now offer an unsurpassed collection of color plates of American freshwater fishes. The original (1908) edition of Forbes and Richardson included 52 species, but in the second edition (1920), 68 species (three appear two or three times) are shown in color. Many of these plates are especially familiar, partly because of reuse in several editions of Hubbs and Lagler (1964). They rank among the best color paintings of fishes known to me. In Smith's work, there are eight new full-page color plates showing 34 species, of which only two (*Etheostoma asprigene* and *E. exile*) duplicate forms shown by Forbes and Richardson. They are also of high quality, though to my eye the reproduction of green seems too bright. Several are the first color paintings of their species to appear. Except for the black crappie on the dust jacket, none of the Forbes and Richardson's paintings appears in color in the new book, but many are reused effectively in black and white. The remaining black and white text figures are either excellent photocopies from the new color plates or new drawings. The latter are clear and functional, but the quality varies and many fall well below the standard set elsewhere.

The principal original contribution of this book is its portrayal of change in the fish fauna through a period of intensive land utilization, burgeoning industry (especially near Lake Michigan and the great rivers), and rapid population growth. Smith has exploited the rich heritage of distributional data from an earlier era (pre-1908, mostly 1880–1905) and compared these, using different map symbols, with new distributional information drawn from many more collection sites in the post-1950 period (mostly 1962–72). The changes, if any, are graphically depicted on the individual maps and are discussed in the species accounts under distribution. Most changes reflect restriction in range in the state (e.g. *Notropis spilopterus*, p. 119) but a few species, tolerant of degradation in water quality (e.g. *Notropis lutrensis*, p. 115), have prospered and extended their distribution and increased in abundance. In an earlier paper (1971), Smith analyzed the effects of human modification on Illinois fishes more fully.

Although one may applaud the mapping approach adopted, ichthyologists will note a disadvantage in the elimination from the maps of the collections taken from 1908–50. True, this was a period of reduced ichthyological activity in the state, but one that included, among others, the major survey of Mississippi River fishes by Barnickol and Starrett (1951), a landmark in major river investigations.

The workmanship in this book is good: the illustrations and typography are clear, the binding is attractive and substantial, and the overall organization is excellent. The proofing is good except for occasional slips such as the misspelling of Sciaenidae (p. 298). Smith deemphasizes the use of pharyngeal dentition in the Cyprinidae, omitting tooth formulae from most keys and even from diagnoses in genera, except *Nocomis* and *Notropis*. Those for *Notropis fumeus* (usually 2,4–4,2) and *N. hubbsi* (usually 4–4) are incorrectly recorded. Commonly encountered and distinctive pharyngeal arches such as those of *Ctenopharyngodon* and *Cyprinus* might well have been described or figured, and the reference value of tooth counts in any cyprinid (e.g. for identification of stomach contents) justifies inclusion. Inevitably, there are some errors in the text, mostly trivial, but these do not detract seriously from the overall excellence of the volume.

Illinois is still among the few states and provinces to boast a top-rated ichthyology. The Illinois State Natural History Survey and the author are to be commended for their support and devotion to this welcome project.

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REEVE M. BAILEY

Museum of Zoology
The University of Michigan
Ann Arbor, MI 48109
USA

(R-255)

Settlement and Metamorphosis of Marine Invertebrate Larvae. *Proc. of the Symp. on Settlement and Metamorphosis of Marine Invertebrate Larvae, held in Toronto, Canada, December 27–28, 1977. Edited by Fu-Shiang Chia and Mary E. Rice. Elsevier, New York. 1978. xi + 290 p. $25.00.*

In marine invertebrates, the form of the larva often differs dramatically from that of the adult. How and where the transition from larva to adult is accomplished is a subject of great interest to invertebrate morphologists and marine ecologists alike. As an extensive literature accrues on larval settlement and metamorphosis, it is generally recognized that settlement processes must be instrumental in shaping adult distributions and abundances. The fate of larval structures and origins of adult ones are important clues to questions of systematics and evolution. This collection of review chapters and research reports presents a useful body of information on this transition in a number of invertebrate phyla.

These proceedings stem from a symposium held in honor of Dr Robert Fernald, Professor Emeritus, University of Washington and Friday Harbor Laboratories. The contributors are his colleagues, students, and students' students. It is a tribute to Dr Fernald that those he has so favorably influenced have produced a sizable portion of the recent work on invertebrate settlement and metamorphosis.

Due, in part, to the extraordinary complexity of invertebrate form, few broad comparisons have been attempted. However, these comparisons can prove to be useful exercises. For example, Hadfield presents a comparison of gastropod metamorphosis, using an opisthobranch example, with that of anuran amphibians. In molecular size and effective dose, the factor which stimulates metamorphosis in opisthobranchs resembles thyroxine, the metamorphosis-inducing hormone of anurans. However, the substance active in opisthobranchs is derived from the environment, specifically coral polyps, rather than an internally secreted substance. The mode of action is very different also; while the anuran hormone stimulates macromolecular synthesis, the action of the opisthobranch effector is more similar to a neurotransmitter. In both cases the morphological events consist of rapid disappearance of the larval structures and subsequent emergence of the adult ones. Whereas the two processes are far from parallel, the comparative analysis establishes a clear context in which to consider the data. Moreover, two areas, the timing and acquisition of metamorphic competence and neural ontogeny, take on new emphasis for research in molluscan metamorphosis. Analyses of this kind can undoubtedly help to reduce the data to experimentally manageable generalizations in other invertebrate groups as well.

The importance of metamorphic events to questions of phylogeny is aptly demonstrated in the chapter by Zimmer on phoronids. He points out that the debate over whether phoronid larvae or adults are oligo- or tri-merous in the same context as echinoderms and hemichordates pivots on the presence and fate of the larval protocoel. His review of current findings on larval anatomy and metamorphosis coupled with the presentation of new comparative data finally set the question to rest. The presence of the larval protocoel is documented in eight of nine species examined; in the ninth species, a transitory protocoel is found in an early developmental stage. In all three species examined through metamorphosis, the protocoel is retained. Therefore, it is safe to conclude that the preoral body regions of larval and adult phoronids are equivalent to the protosome of tri- or oligo-merous deuterosomes.

It is generally agreed now that the great diversity of form and the variety of mechanisms of metamorphosis rule out broad generalities which are useful across many phyla. However, in his concluding remarks, Chia presents a general model of the control of metamorphosis which connects environmental cues and morphogenetic events. Although the model provides a basis for discussion, so little is known about the physiology of metamorphosis that no likely candidates come to mind for the various internal chemical effectors proposed. In fact, evidence is not available to support the assumptions of the model; for example, the existence of a simultaneous control for both the loss of larval structures and the development of adult ones. This discussion does point out two interesting generalities borne out by existing data: (1) Adults with general diets should employ less specific cues to settlement than those with restricted diets. (2) Gregarious and sedentary species are more dependent on environmental cues for settlement than nongregarious and mobile ones.

The various chapters complement each other well. The report of new data on vesicularioid bryozoan settlement by Reed fits well with the clearly written review of cellularioid metamorphosis by Woollacott and Zimmer. Coelenterate settlement and metamorphosis by Chia and Bickel lays the ground for Otto's report on the settlement of a stauromedusan. As a whole the chapters by Hadfield, Bonar, and Switzer-Dunlap provide full coverage of opisthobranch settlement and metamorphosis. While Hermans, Potswald, and Eckelbarger detail these processes in three polychaete families, a general review of polychaetes is lacking. The combination of Chia and Burke on sea urchin metamorphosis and Strathmann on echinoderm settlement covers the existing literature thoroughly.

In all these pieces, the authors present current findings and often contribute original data (for example, the chapter on turbellarians by Rupert). In addition, a number of unpublished theses are brought into wider circulation by reference. However, the taxonomic coverage is uneven. Reviews by Pilger on echiurans and Rice on sipunculans complete the consideration of spiralian groups. Among the molluscs, the emphasis is on opisthobranch gastropods with little mention of prosobranchs and no space is devoted to bivalves. Barnacles, especially parasitic forms, are fully described by Lewis, but no other crustaceans are discussed. To some extent, the coverage reflects the existing evidence; however, a sizable body of information exists for several of the omitted groups.

As befits a publication on morphology, the book is amply supplied with excellent line drawings and photographs. The light micrographs of whole-mounted larvae, which are technically difficult to obtain, are a particular highlight.

In conclusion, these proceedings are a timely collection of useful information for students and investigators. It will undoubtedly become a prominent feature of a general reference collection on invertebrate reproduction and development.

R. ANDREW CAMERON

Division of Natural Sciences
University of California
Santa Cruz, CA,
USA

(R-262)

ANNOUNCEMENTS/AVIS

Workshop on Periphyton in Freshwater Ecosystems

The Institute of Limnology, University of Lund, will host a limnological workshop on periphyton in freshwater ecosystems in Växjö, Sweden, September 14–17, 1982, held under the auspices of the Societas Internationalis Limnologiae.

The scope of the workshop is to evaluate the complex structure, dynamics, and functional significance of periphytic organisms of lake and stream ecosystems. While the emphasis will be directed to attached bacteria and algae, the interactions of micro- and macro-fauna upon periphyton will also be treated.

Organizing Committee: R. G. Wetzel, USA; S. Björk, Sweden; and J. Barica, Canada.

For further information write to: *Robert G. Wetzel, Program Chairman, W. K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, USA.*

Atelier sur le périphyton dans les écosystèmes des eaux douces

L'Institut de limnologie de l'Université de Lund organisera un atelier sur le périphyton dans les écosystèmes des eaux douces à Växdö (Suède), du 14 au 17 septembre 1982, sous le parrainage de la Societas Internationalis Limnologiae.

L'atelier a pour objet d'évaluer la structure complexe, la dynamique et l'importance fonctionnelle des organismes du périphyton des écosystèmes des lacs et des cours d'eau. Les participants s'attacheront avant tout à étudier les bactéries et les algues connexes mais traiteront également les interactions de la faune microscopique et macroscopique sur le périphyton.

Comité organisateur: R. G. Wetzel (É.-U.), S. Björk (Suède) et J. Barica (Canada).

Pour renseignements, écrire à: *Robert G. Wetzel, chargé de programme, W. K. Kellogg Biological Station, Michigan State University, Hickory Corners (MI) 49060, É.-U.*

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from northeastern Grand Bank **I-H. Ni**

Acclimation to copper by rainbow trout (*Salmo gairdneri*) — a modifying factor in toxicity **D. G. Dixon and J. B. Sprague**

Abiotic versus biotic uptake of radiophosphorus in lake water **S. J. Tarapchak, D. R. Slavens, and L. M. Maloney**

Patterns of invertebrate size and abundance in aquatic habitats **G. G. Mittelbach**

Effect of the algal length/aperture length ratio on Coulter analyses of lake seston **H. A. Vanderploeg**

Uptake of cadmium in perfused rainbow trout (*Salmo gairdneri*) gills **P. Pärt and O. Svanberg**

Culture à dialyse: emploi de fibres creuses dialysantes pour la culture massive de phytoplancton **P. Marsot, R. Fournier et C. Blais**

Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*) **C. A. Busack and G. A. E. Gall**

Determination of mineral fiber concentrations in fish tissues **A. R. Batterman and P. M. Cook**

Establishment of walking catfish (*Clarias batrachus*) cell lines and development of channel catfish virus vaccine **E. J. Noga and J. X. Hartmann**

Fate of Kepone and mirex in a model aquatic environment: sediment, fish, and diet **D. R. Skaar, B. T. Johnson, J. R. Jones, and J. N. Huckins**

Reproductive uncertainty and the evolution of iteroparity: why don't flatfish put all their eggs in one basket **D. A. Roff**

Relationships between nannoplankton and lake trophic status **S. Watson and J. Kalff**

Release of phosphorus by certain benthic invertebrates **W. S. Gardner, T. F. Nalepa, M. A. Quigley, and J. M. Malczyk**

A method for comparing the precision of a set of age determinations **R. J. Beamish and D. A. Fournier**

Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen **R. V. Thurston, G. R. Phillips, R. C. Russo, and S. M. Hinkins**

Abundance, like history, and growth of introduced populations of *Mysis relicta* in subalpine California lakes **M. D. Morgan**

Absorption of selected antimicrobic drugs from water by channel catfish, *Ictalurus punctatus* **K. E. Nusbaum and E. B. Shotts Jr.**

Absence of the protozoan *Myxosoma cerebralis* (Myxozoa: Myxosporea) the cause of whirling disease, in a survey of salmonids from British Columbia **L. Margolis, T. E. McDonald, and G. E. Hoskins**

Determination of free metal ion concentrations using bioassays **V. Borgmann**

On estimating partial recruitment in virtual population analysis **D. A. Roff**

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